

Pitfalls for National Science Foundation

THE National Science Foundation occupies a central place among scientific institutions in the United States, which is why its influence is necessarily greater than the mere size of its budget would suggest. What has happened in the past few months in Washington suggests that the National Science Foundation has taken the measure of its own importance, which is a step in the right direction. There are also signs, however, that the foundation is beginning to stray from the straight and narrow path along which the interests of basic science in the United States must lie. Both at the hearings before the subcommittee on Science Research and Development two weeks ago and from what officials of the foundation say about their intentions for the next few years (see page 348), it is plain that important innovations have been embarked on. Now is the time to ask whether they make sense.

The longest shadow will be cast by the new policy on supporting graduate students at colleges and universities. The foundation has reconciled itself to the policy it accepted with a show of reluctance last year of doing away with traineeships awarded to graduate schools and used for supporting selected students, restricting the number of fellowships awarded competitively to 500 each year and dispensing altogether with grants to institutions, usually university departments, where there seems to be a need either to strengthen or to preserve an organization capable of contributing in an important way to the output of PhDs.

The foundation has in the process turned away from the principle established in the years immediately after the flight of the first Sputniks that the resources of the federal government should be used to train large numbers of highly qualified scientists and engineers. To be sure, it will still be possible for aspiring graduate students to be carried on the backs of those applying for research grants, but it seems openly to be acknowledged that one result of such a dependence will be to favour the large state universities at the expense of the private universities on the one hand and the universities with immature or even non-existent graduate schools on the other. There are also dangers in the way in which the new system will require graduate students to depend for the continuation of their work either on the success of their supervisors in getting grants renewed or on the necessarily straitened funds of universities. In the circumstances, it is for the foundation to show that its change of policy is wise.

Most explanations of the change of course include some reference to the labour market for PhDs, and it is true that these are difficult times for fitting some kinds of PhD graduates into jobs that seem to them to be acceptable. The trouble, in this and other fields, is that the labour statistics are probably misleading and certainly irrelevant. For one thing, the incidence of unemployment among PhDs is probably much less than the news

of hardship among physicists, for example, would suggest. (It is in any case disingenuous of the Administration to feign surprise at a development which is in part at least a consequence of government policies towards the aerospace industry among others.) Second, there is no reason to suppose that present difficulties imply permanent surpluses. A resurgence of the United States economy could replace surplus by shortage within a very few months. The lead time in the production of PhDs is so long that there is even a case for thinking that when there is an apparent shortage, the Administration's influence should be used to ensure that recruits are not frightened away from the graduate schools. Third, it is more than probable that some of the difficulties of placing PhD graduates stem from too narrow a view of the jobs which they can usefully and respectably take on, which in turn implies that the shortage could go away as the labour market becomes more flexible and imaginative. To be sure, there may be much in what the National Science Foundation in the United States and the Science Research Council in Britain are saying about the need for alternative courses to the conventional PhD, and this is one thing on which the universities should concentrate their attention.

Where the justification of the National Science Foundation's new policy is concerned, however, what matters is not the threat of over-production of PhDs, real or imaginary, for, after all, even in its heyday the foundation has supported only rather less than 10 per cent of graduate students in science and technology by means of traineeships. Keeping on with them would not in itself deny the foundation an opportunity to balance supply against demand, for there are still handy regulators in the alternative methods of keeping on promising graduates as research students. The foundation's support for research assistants through its research grant programme is one obvious method of control. Where the new policy will make a difference is in the ease with which graduate schools are able to emerge in places where they do not exist at present.

So is it that the foundation has decided that there are already too many universities with ambitions of this kind? Something like 200 out of 2,000 American universities are at present providing graduate education on a substantial scale and most policies in the past few years have been founded on the assumption that this is too narrow a base for comfort. This, for example, was one of the points made eloquently two years ago by the National Science Board in its statutory report to Congress. What has happened since to change the situation? How can the Administration be sure that its new dispensations will not increase the difficulties of recruiting good teachers to out of the way places? Is there not a danger that in the new arrangements, it will be harder than ever for the graduate schools to aspire to the diversity which



is desired and probably desirable? Will not the change of policy reinforce the dominance of the large state universities in the pattern of graduate education in the United States? Above all, is there not a serious risk that the new system will limit the capacity of the universities to provide advanced training for the large numbers of students almost certain to be required by the late seventies? The foundation would be in a better position to shrug off the last question if it had been more diligent and more expert in making the manpower studies which are plainly necessary. On the face of things, it is taking a great risk.

Much the same is true with the foundation's programme of applied research, another important innovation for 1972. The danger here is that the foundation may have bitten off more than it can comfortably chew. To be sure, everybody agrees that it would be a splendid thing if science and technology could be harnessed to what have now been called "national needs". The problem is that wishes do not easily become realities. In the past few years, for example, there has been a great deal of disappointment in the implicit promise that science and technology automatically bring prosperity as measured by the rate of growth of the GNP. Is there not now a danger that many of the programmes being mounted by the National Science Foundation will convey the implicit promise that national needs of particular kinds will similarly be satisfied? The programme of meteorological research will almost certainly be fruitful, for example, if only as a means of keeping other agencies (such as the newly created National Oceanographic and Atmospheric Agency) on their toes. But is there not a danger that by marketing the project as one that is somehow linked with the possibility of controlling climate, the foundation may be giving hostages to fortune? The other projects on the foundation's shopping list at present are similarly well constructed, but here again there is a danger that too much may be expected of them. If

anything, the name chosen for these activities as a group—Research Applied to National Needs—is too grand for comfort. Would it not be prudent for the foundation to back-pedal gently on this issue?

Another continuing source of anxiety is the distribution of resources. Over the years, the foundation seems constantly to have been tempted to spend its funds on schemes not strictly relevant to its primary objectives. Even if the new budget comes true, less than a half of what the foundation spends will go on the support of research in universities. The new programme of applied research promises to be one of the fastest growing in the foundation's pattern of activity. The obvious danger is that it will turn out to be a diversion of resources from the overriding task of making the fullest use of the intellectual resources of the United States. Only time will tell, but this is one of the points at which the foundation's critics will direct the closest attention.

The foundation's credibility would be more easily assured if it were somehow able to carry greater weight. The appointment of four distinguished scientists as assistant directors has not by itself removed the fuzziness which characterizes the dealings of the foundation with the outside world. In comparison with the National Institutes of Health, for example, the foundation gives the appearance of being less confident of its internal machinery. Certainly the study sections of the NIH, responsible for awarding research grants, have a prestige to which the corresponding committees of the NSF have hardly begun to aspire. And it remains a painful fact that after several years of promises about the development of manpower statistics, the foundation is still at a loss to know the full extent of the supposed surplus of PhDs. Sceptics outside must inevitably be alarmed that the growth of the foundation's budget will not be matched by the growth of its capacity to deal with day to day business. This is another point to keep the management awake at night.

When to Drop Out

THE Confederation of British Industry will have made few friends in government laboratories with its somewhat patronizing suggestions last week as to how the careers of government scientists should be modified. To be sure, what the research and technology committee of the CBI has to say is sensible enough. The difficulty is that, first of all, there are ways in which government scientists may already seem at a disadvantage with their colleagues in industry. Second, the issue of how the careers of scientists and engineers in government service should be formed is only a part of the very much larger question of how governments should seek to exploit scientific resources as a whole. Third, on the principle that those who live in glass houses should not throw stones for fear of reprisals, there are many defects in the career structure of scientists in industry which resemble those to be found in government service, and many reasons to believe that similar remedies should be applicable.

A part of the CBI's complaint about the present situation in government laboratories is that because of

the regular increments of salary that government employees enjoy and the lack of temptations from the straight and narrow path of scientific research, government scientists and engineers tend to be at once older and less alert than those in industry, or so the CBI considers. One simple escape from the difficulty is the suggestion that there should be a more flexible approach to employment of scientists and engineers in government service and that people should be employed on short-term contracts rather than in perpetuity. The trouble with this, of course, is that it sounds like a prescription for bringing almost to a halt recruitment of scientists to the government service. Few other employers would have the gall to offer short-term appointments to qualified people precisely because of a belief—right or wrong—that technical people are likely to be less useful as they grow older.

So what should be done? The first thing to say is that the commission on the Civil Service under Lord Fulton put forward a great many proposals which have not yet been implemented. In general, Fulton would have created

many more opportunities for lateral movement within government service that would be expected to carry scientists outside the narrow practice of research and development into administration and management. This is not merely a way of saving the careers of technical people in government service but also a source of the

technical skills which the rest of the Civil Service needs. It is a great misfortune that so little has been done in the years since the Fulton commission reported to put flesh on this skeleton. It is another misfortune that the CBI committee has made too little of those opportunities.

Taxation and Women's Liberation

MR ANTHONY BARBER, the Chancellor of the Exchequer in Mr Heath's new British government, is unlikely to be mistaken for a social radical of the kind likely to be found marching with the Women's Liberation through the streets of London. It is therefore a little ironical that, in his Budget last week, he should have taken one of the most significant steps for several years towards meeting the demands of those who consider that women are at present unfairly treated in British society and that something should be done to redress the balance. Last Tuesday, Mr Barber announced that from April 1972, women who work will not have to suffer the indignity of having their earnings aggregated with those of their husbands for taxation purposes. Gone in one step will be the old British joke that cohabitation without marriage is an effective means of income tax avoidance. The interesting question, for women in the Women's Liberation movement as well as for their husbands, is whether Mr Barber's innovation will make much difference to the frequency with which married women at present go to work and to the way in which they are regarded when they get there.

Although Mr Barber's incentives are likely to carry weight with many women, it is unlikely that they will satisfy the Women's Liberation or, more important, substantially improve the chances that women will find it possible to go out to work in greater numbers. As any aspiring Kate Millett will find out, there are practical as well as financial obstacles to be overcome. The most obvious is that women who wish to combine careers and children often find it necessary to put the children first. It is not merely a question of having to stay at home for measles. Much more important is the now well demonstrated truth that young children up to four or five need more of some parent's attention than the full blooded pursuit of a profession will allow. The Women's Liberation is probably right in asking that there should be better facilities for caring for children during the day (and Mr Barber's Budget may make this easier by simplifying payment for day nurseries) but that is only a small part of the battle. The most serious difficulty is that no amount of enlightened public administration will help to banish the awkward fact that working women are likely for a long time to be more severely handicapped than their husbands by the need to care for children. The question is not whether to ignore this awkward truth but how to make its influence less obviously a source of social injustice towards women.

What is to be done? If the Women's Liberation movement were prepared to take a level-headed view of its responsibilities, it would first of all concentrate on demonstrating that responsibility for child care is not nearly as serious a handicap as employers frequently pretend. To be sure, there are inevitable risks in taking,

say, five years off in the middle of a career as a ballet dancer or a pianist. With most kinds of intellectual work, however, the damage done by interruption is not nearly as important. Moreover, it can often be arranged that women carry on with part-time work while looking after children. Over a period of years, women must often be more diligent and more constant employees than men, who more often flit from place to place in pursuit of their careers. In all the circumstances, what the Women's Liberation should and could now undertake is to exploit the potential benefits of Mr Barber's concession by a shrewd demonstration of the potential advantages of working women. Such an undertaking would be important not merely for the women concerned but for all who are alarmed at the waste of talent unemployed.

100 Years Ago



The principal reason why there has been greater scope for the improvement of the steam engine than for the electro-magnetic engine arises from the circumstance that in the formula $\frac{a-b}{a}$, applied to the steam engine by Thomson, in which a and b are the highest and lowest temperatures, these values are limited by practical difficulties. For a cannot easily be taken above $459^{\circ}+374^{\circ}=833^{\circ}$ from absolute zero, since that temperature gives $12\frac{1}{2}$ atmospheres of pressure, nor can b be readily taken at less than the atmospheric temperature or $449^{\circ}+60^{\circ}=519^{\circ}$. Also there is much difficulty in preventing the escape of heat; whereas the insulation of electricity presents no difficulty,

I had arrived at the theory of the electro-magnetic engine in 1840, in which year I published a paper in the 4th Vol. of Sturgeon's Annals, demonstrating that there is "no variation in economy, whatever the arrangement of the conducting metal, or whatever the size of the battery." The experiments of that paper indicate 36 ft. lbs. as the maximum duty for a grain of zinc in a Wollaston battery. Multiplying this by 4 to bring it to the intensity of a Daniell's battery, we obtain 144 foot lbs. Here, as in the experiments in the paper on Mechanical Powers of Electro-Magnetism, Steam, and Horses, the actual duty is less than the theoretic; which is owing partly to the pulsatory nature of the current, and partly also to induced currents giving out heat in the substance of the iron cores of the electro-magnets; although these last were obviated as far as possible by using annealed tubes with slits down their sides.

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OLD WORLD

FURTHER EDUCATION

CNAA Success Story

by our Education Correspondent

SOME idea of the impact made by the Council for National Academic Awards on the colleges of further education can be gleaned from the latest statistics published by the Department of Education and Science. There are now more students in the further education sector following degree courses approved by the CNAA than there are studying for external degrees of the University of London. In 1969, for example, these figures were 18,300 and 13,500 respectively (*Statistics of Education, 1969, Vol. 3 Further Education, HMSO, £1.40*). The CNAA has therefore firmly established an alternative form of higher education in Britain in less than a decade.

But the impact of the CNAA has been even more far-reaching than the mere numbers of students suggests. The council's function is to approve courses submitted by non-degree awarding institutions, and to ensure that their standards are equivalent to those in traditional universities. The non-university sector of British education therefore now has an open pipeline leading to degree status other than the already overloaded external degree system of the University of London. Moreover, many of the CNAA approved degree courses are more radical than their counterparts in the universities and from its inception the council has smiled on the sandwich course method of study.

The DES statistics tell more, however, than the success story of the CNAA. In 1969, for example, there were more than three million students enrolled in grant-aided further education establishments—a figure which puts in exceptionally bad light the lack of attention that has traditionally been paid to this sector of education. Of the total, 88 per cent of the students engaged on full-time and sandwich courses, 83 per cent on part-time day courses and 40 per cent on evening courses were working for recognized qualifications, the bulk of these qualifications being lower than GCE A-level.

A noticeable trend in the further education sector during the past few years has been the relative and at times absolute decline in the numbers of students engaged on part-time and evening courses. Between 1961 and 1969, for example, the numbers following full-time and sandwich courses leading to advanced qualifications increased at an average rate of nearly 17 per cent a year, while those engaged on part-time courses increased yearly by 7 per cent

and evening course enrolments remained almost static during the decade. A similar trend also took place in the lower level courses, with the numbers of full-time students, part-timers and evening students increasing yearly by 11.6 per cent, 5.5 per cent and –1.0 per cent respectively.

In spite of the fact that the statistics are two years old, their publication by the DES is a particularly useful exercise. It is one of the few attempts to draw together information about this important but little discussed sector of education which includes the polytechnics, colleges of technology, technical colleges, colleges of art and agricultural colleges—establishments whose only common denominator is often the fact that they all fall directly under the control of local authorities rather than the DES.

FRANCE

Clarifying Proposals

Paris, March 29

MORE weight was added to the rising tiers of French scientific bureaucracy when on March 29 the Ministry of Industrial and Scientific Development announced the creation of yet another liaison department, the Service des Programmes des Organismes de Recherche (SEPOR). This new unit will serve as the link between the various research organizations which at present are administered directly by the Ministry, the Centre National pour l'Exploitation des Océans (CNEXO), the National Space Research Centre (CNEX), the Applied Data Processing Research Institute (IRIA) and the Atomic Energy Commission. The new department will be run by Mr Maurice Levy, a theoretical physicist who recently served for two years as scientific counsellor to the French Embassy in Washington.

The task of SEPOR will be to "put into clear text" scientific programmes concerning, primarily, oceanological and space research for the minister, Mr F. X. Ortoli. It seems that Mr Ortoli has lately experienced some difficulty in understanding the exact significance of some of the research proposals which have been put forward in these fields. SEPOR may well find itself in conflict with the Délégation à la Recherche Scientifique et Technique (GGRST), the inter-ministerial unit headed by physicist Pierre Aigrain, which is directly responsible to Ortoli and which carries the responsibility for coordinating the activities of the National Scientific Research Centre (CNRS), the Health and Medical Research Institute (INSERM) in addition to the space and oceanographical agencies.

The somewhat equivocal position which the oceanographical centre,

CNEXO, occupies in the French scientific establishment was well illustrated by the programme of the international colloquium on the development of the seas, organized by CNEXO and held in Bordeaux in early March. The centre seems to represent a supplement to the various oceanological efforts in the public and private sectors rather than taking the initiative as a coordinating organization. In effect, CNEXO has arrogated to itself the role of the Centre National de Recherches Scientifique (CNRS) in the field of basic oceanographical work, yet aims the thrust of its marine research towards the needs of the petroleum industry—which pays the bill—and this without the assurance that petroleum orientated research is necessarily the most profitable. This could be an unfortunate trend—until now, French oceanological research has not been at all bad.

POLLUTION

Guillemots and PCBs

THE accusing finger which pointed towards environmental pollutants as the cause of death of thousands of seabirds in the Irish Sea during the autumn of 1969 may have been a little premature, but it seems that at least one enlightened manufacturer is taking no chances.

Although records of "wrecks", particularly of the larger auks, go back to the middle of last century, long before pollution could be incriminated, none has been so devastating as the Irish Sea disaster which claimed at least 12,000 birds, mostly guillemots (*Nature*, **224**, 300, 402; 1969).

With a commendable sense of urgency, the Natural Environment Research Council stepped in to coordinate what has proved to be perhaps the most extensive and concentrated investigation of an incident of this kind yet undertaken. The work culminated in a report and supplement edited by Dr M. W. Holdgate, who at the time of the investigation was Deputy Director (Research) of the Nature Conservancy. This report is summarized in a booklet published by the NERC last week (NERC Publication Series C No. 4).

Nearly all the birds were suffering from malnutrition. The breast muscles were atrophied, and subcutaneous fat had been completely mobilized, but the most interesting and controversial finding was of unusually high concentrations of polychlorinated biphenyls (PCBs) in the livers of the majority of the birds, concentrations much higher than had previously been detected in land or fresh water animals. Whether the lesions in the liver caused by the PCBs were sufficient to cause the death of the birds remains conjecture, but is unlikely. Analysis of the livers of ap-

parently healthy birds shot in the area after the disaster demonstrated similarly high PCB concentrations. It is of great interest, however, to discover that PCBs, which are lipid soluble and normally dispersed throughout subcutaneous and other fat reserves, become concentrated in the liver when this fat is mobilized during periods of undernourishment, and although not a primary cause of the Irish Sea "wreck" may have been a contributory factor to the lowering of resistance of a large number of birds already weakened by the stresses of the moult. What initiated their undernourishment remains unknown, but the cause could have been a natural disaster, possibly an undetected failure of the food supply or a disease-initiated interruption of their normal feeding habits.

It seems that several different factors in combination were responsible for the disaster. If this is the case, then pollutants could be more insidious than the lethal dose figures would have us believe, for even in relatively low concentrations, they may be sufficient to tip the scale of the balance between life and death of an animal already under some form of natural stress.

For those who will look on this report as merely perpetuating the use of PCBs as the latest in a chain of toxic scapegoats, a recent announcement by Monsanto Chemicals Ltd, the sole manufacturers in Britain and the USA, should cause them to question their philosophy. Without any announcement, or, it seems, documented justification, Monsanto have placed heavy restrictions on the sale of PCBs in Britain, following a similar restriction imposed on US sales several months ago. Their Newport factory is also to significantly reduce production of these persistent and highly toxic industrial chemicals.

COMPUTER POLICY

Mediator Snags Aired

MEMBERS of the Select Committee on Science and Technology have been eager to investigate the continuing criticisms of the Linesman/Mediator air traffic control system ever since their investigation of the British computer industry began last year. Not only is the Linesman radar data processing system that is at the heart of the system one of the largest computer complexes produced in Britain, but together with the Mediator system that is primarily concerned with the processing of flight plans, it will look after both air defence and air traffic control in Britain. Last week members of the Select Committee got their chance when the Ministry of Defence gave evidence to subcommittee A. In a memorandum to the subcommittee, the

Ministry of Defence admitted significant delays in the project, and it seems that the cost of the system has risen from the original £106 million to £145 million.

Partly civil and partly military, the Linesman/Mediator system is based on three air defence radar stations and nine air traffic control radar stations, which feed information into a control centre at West Drayton for both air defence and civil air traffic control. The system is being built up in stages. It seems that the chief problems are to do with the software; and these are being met both in the Mediator part of the system to do with the processing of flight plans, and in the Linesman system for the processing of the raw radar data.

But the system is progressing, according to the memorandum. Simulators at West Drayton for training purposes are installed and are now being programmed, the height finding computers and anti-jamming computers at the individual radar stations are installed and partially programmed, the computers for local control by air defence radar stations are in production, hardware for the flight plan processing system is being installed and programmed, and various data processing elements for the radars are either in operation or in production.

The Ministry of Defence agrees that some of the techniques in the system are obsolete by now, but points out that this was bound to happen in a system that is going to take a long time to develop. And almost every large project involving real time on-line computers has met serious difficulties in planning the design and in the preparation of the software. But the subcommittee nevertheless did not appear entirely happy.

As the largest user of computers in the government sector, the views of the Ministry of Defence were also sought on other aspects of the computer scene. According to the memorandum the present policy of the ministry towards computers for weapons systems is to use general purpose computers derived from business machines wherever possible. The ministry also expressed concern about what it calls "a heavy NATO investment in US hardware and software in the higher echelons of command", a tendency which the other nations are finding increasingly hard to resist. The hope is that a joint civil and military body called the NATO Command, Control and Information Systems and Automated Data Processing Committee, and helpfully abbreviated to NCCDPC, will help to counteract this trend. One of the duties of this body, on which Britain is represented, will be to advise on the choice of hardware and software for NATO systems after tenders have been received.

Parliament in Britain

Vivisection

MR KENNETH LOMAS is appalled by the number of experiments carried out on living animals each year; he pointed out that in 1960 the number of animals used in such research was about 3½ million, and the number of investigators who could legally perform such experiments was about 7,000. By 1969, however, the number of experimenters had risen to 13,791, and the number of animals involved was 5,418,929, or 10 animals every minute of the day and night, seven days a week. He questioned whether all these experiments were really necessary, and suggested that there is a need to establish a research institute, under the aegis of the Medical Research Council, to study and develop methods of medical research which would not involve living animals. The National Anti-Vivisection Society, he said, had estimated the cost of such an establishment to be about £1 million, including running costs for the first 5 years.

Mrs Margaret Thatcher, Secretary of State of Education and Science, was not moved by Mr Lomas's rhetoric. She emphasized that animals must be used for testing drugs and food where the use of human subjects would be hazardous, and that a wide range of advances in modern medicine would not have been possible without the use of animal subjects. (Debate, March 31.)

Desalination

IT seems that there is no immediate prospect of a contribution to Britain's water supply from desalination. Mr John Farr asked how many desalination plants might be operating in Britain by December 1972. Mr R. Graham Page, Minister for Local Government and Development, said that none would be functioning for the supply of water to the public. Information about the cost and the feasibility of using such plants to supply water for public use would be available in three to four years' time through experience gained from the experimental freezing plant at Ipswich, due to open in two years' time. (Written answers, March 31.)

Excavation

THE fuss which "Rescue", the archaeological group, made over Cublington may have struck home. Mr Andrew Faulds asked if an archaeological survey will be made a compulsory condition of planning permission in future development schemes. Mr R. Graham Page, however, answering for the Secretary of State for the Environment, felt that there was no need to extend the protection already provided by the Ancient Monuments Acts. (Written answers, March 30.)

NEW WORLD

New Brooms begin to Sweep

from our Special Correspondent

Washington, April 5

THE Administration is beginning to talk about the administration of science and scientific institutions more confidently than at any time in the past two years. The arrival of Dr Edward David at the White House as Science Adviser to the President has plainly helped to clear a good many minds. It also helps that the National Science Foundation has begun to grow at long last, even though much of the conspicuous growth in the Budget for 1972 consists of expenditure that would previously have been incurred by other agencies. But the clarification of objectives has also made it possible for disagreements to be more easily identified. If the new broom at the White House is indeed a sign of more effective administration, it is inevitable that it will also bring sharper disagreements.

The first issue is the Administration's relationship with graduate education in the United States. With this year's budget, the National Science Foundation seems finally to have turned its back on generalized support of research students at American universities and of university departments which may grow into graduate schools of distinction. Thus the Administration has repudiated the pleas of the National Science Board two years ago that graduate education in science and technology should be recognized as a national responsibility. So much is clear from the way in which, a week ago, the new chairman of the National Science Board, Dr H. E. Carter, pleaded for more practical objectives in basic research and quite ignored the contentious issue of graduate education.

Dr William McElroy, the director of the National Science Foundation, said in an interview this week that he has indeed been persuaded in the past twelve months that there should be a change of policy, at least for the time being. "I don't feel as strongly as I did last year." The biggest change, he said, has been in the relationship between supply and demand for graduate students. At times when it seems that graduate schools are turning out more graduates than the economy can absorb, traineeships for graduate students are neither necessary nor desirable.

He did, however, admit that the abandonment of traineeships would affect the good private institutions more seriously than the large state universities in which graduate schools have

flourished in the past few years. Dr McElroy thought it likely that graduate enrolments at the private universities would be decreased by ten per cent or perhaps a little more. He argued that the new system of carrying traineeships on research grant budgets and the abandonment of institutional grants would make it easier to encourage junior people in university departments.

But how much reliance should be put on the employment statistics? Dr McElroy pointed out that the picture is patchy. "We only heard of unemployment when the economy turned round." Then some disciplines are more severely affected than others. "New engineering PhDs have no trouble." One difficulty is that of collecting reliable statistics and the National Science Foundation is hoping to glean something about the pattern of employment among graduates in science and technology with the help of alumni organizations in American universities. He also said that the foundation will be prepared to take another look at its new policy of graduate support if there should be signs of change in the labour market or if accurate statistics showed that the present policy is misguided.

This view is entirely in tune with that of Dr David, who said this week that the question is not so much whether the government has an obligation towards graduate education but which method of financing graduate students would be best. The Administration's view is clearly that the present system will make possible more sensitive control of the output of PhDs although, here again, it seems clear that accurate statistics are a necessary and still absent ingredient of policy.

The Administration is also looking for qualitative changes in graduate education. Dr McElroy wants a closer coupling between industry and the universities, more awareness in the universities of the ways in which science and technology can be applied to practical problems—"socially relevant—I guess that's the word we must use"—and greater reliance in the universities on new methods of instruction.

The listener can detect signs of exasperation with the universities. Dr McElroy takes the line that conventional PhD courses are likely to be suitable only for about 15 per cent of the entering class, and that there should be devices for tempting students into other kinds of advanced degrees

not necessarily involving research. He also wants to see a greater diversity of entrants for the universities as a whole—an echo of the Newman committee's report (see *Nature*, **230**, 197; 1971). "In some way the academics have gotten too isolated in their ivory towers."

The foundation's research budget could become another bone of contention in the months ahead. Although (if Congress gives the foundation what the Administration has allowed it to ask for) the budget for 1972 will be an increase of \$110 million, or 21 per cent, expenditure on research will increase more sharply. Thus research projects will have an extra \$86 million or 45 per cent more in the coming financial year. The question is how much of this increase is accounted for by the transference of implied obligations from other parts of the Administration. Dr McElroy guesses that the real increase in the research budget, which includes such things as the cost of the Materials Research Laboratories transferred from the Advanced Research Projects Agency, is more like 10 per cent than 45 per cent. Elsewhere, moreover, the budget includes the cost of naval support for the Antarctic research programme.

So is there a danger that even the larger budget will leave obvious defects unattended to? Dr McElroy agreed that the lack of funds in the new budget for a new radio telescope was one sign of trouble. "We must start worrying about our facilities. It's our feeling that a lot of things are getting pretty old . . . Half our oceanographic research vessels are more than 25 years old and some of these are anyway just conversions . . . We are rapidly getting into trouble with in-shore facilities." He went on to say that under present circumstances, the foundation rarely provides equipment costing more than \$10,000 and that future budgets would have to make up for this deficiency.

One of the foundation's new departments is the programme called Research Applied to National Needs, an attempt to apply the foundation's funds to the solution of practical problems of a technical character. The new budget suggests that spending on this kind of work will increase from \$33 million in the current year to \$81 million in 1972 and Dr McElroy said that projects included in this plan would by

their momentum require an extra \$25 million in the following year. He explained this week that the emergence of the RANN concept is a radical change of policy.

For one thing, it means importing into the foundation's research some of the techniques of management hitherto found in NASA and the Department of Defense. The RANN programme also raises novel problems in the relationship between the foundation and other agencies of government. Dr McElroy hopes that it will be possible to iron out any differences that emerge in the committee of the Federal Council on Science and Technology that will be established for the purpose. Dr David, as sure as anybody that the policy is a new departure, said this week that he hopes the RANN projects will become devices by means of which the mission agencies are given a serious and continuing interest in basic research.

Against this background, there is evidently plenty of scope for continued expansion at the NSF. Dr McElroy is especially hopeful that the foundation will be able to carry out some of the basic research and development on the biological insecticides as well as on the development of new sources of energy. He wants to see the foundation's international connexions strengthened, and already there is talk of how to build a thoroughly international accelerator for the high energy physicists to succeed the generation of machines now being built. Next year, Dr McElroy thinks, the foundation will be in a position to ask Congress for \$850 million. Evidently it is well on the road to the goal of a billion dollar budget, which is no doubt why the Congressional benches were full at the public inquiry into the budget last week.

ENVIRONMENT

Detergers Deterred

by our Washington Correspondent

THE merry game of harassing unruly manufacturers for the good of the environment is an excellent new sport but one that needs some Queensberry rules. Shooting down the SST was a noble deed, but the price of glory to the American taxpayer when the last cancellation charges have been paid will approach \$1,000 million. The Department of Health, Education and Welfare allowed the use of cyclamates to grow unchecked, despite several cautions from the National Research Council, throughout the 1960s, and then, in a fit of convulsive self-reversals, ended up by banning the sweeteners altogether on demonstrably fallible evidence — a splendid performance for those who relish bureaucratic somersaults but bad news for manufacturers with ware-

houses full of canned products containing cyclamates.

Detergent manufacturers are the latest victims of environmental roulette as played in Washington.

In some ways the manufacturers invited the trouble by their advertising methods, which for years have swamped the media with a deluge of frothy hyperbole. Miracle washdays no longer dawn so bright. The detergent manufacturers are now being mercilessly harried by local action groups in the United States and Canada, by the Department of Health, Education and Welfare, by the Council on Environmental Quality, by the Environmental Protection Agency and by committees in both houses of Congress.

The hue and cry was of course first raised by the indications that phosphate, a principal ingredient of most detergents, is responsible for the eutrophication of lakes. As the environmental movement gathered strength, the phosphate content of detergents became an attractive target on which conservationists might demonstrate their zeal. The detergent makers argued that there was no substitute for phosphate in detergents, that America's lakes and streams were too far gone for any change in detergent composition to help, and alternatively that only the construction of advanced sewage treatment plants could help prevent eutrophication.

These arguments fell on stony ground, not least because the manufacturers were known to be developing an apparently harmless substitute for phosphates known as nitrilotriacetic acid or NTA. Then the blows began to fall thick and fast. On April 9 last year, the Committee on Government Operations of the House of Representatives issued a report recommending that all phosphorus be eliminated from detergents within two years. The Canadian Government banned all phosphate detergents beginning from December 31, 1972. Last October, the City Council of Chicago embargoed all detergents containing phosphates from mid-1972, limiting phosphate content to 8.7 per cent by weight in the interim. Residents of Suffolk County, New York, tired of having to blow a head of foam off their tap water before drinking it, banned the sales of almost all detergents whether containing phosphates or not.

The tide of public sentiment against phosphates had long been foreseen by the manufacturers, and with apparently no discouragement from the government they had begun putting their money into NTA. Almost the only source from which a clear warning was enunciated was the Senate Public Works Committee. A study prepared for the committee by Dr Samuel S. Epstein of the Harvard Medical School gave warning last November that NTA

should not be substituted for phosphate until its environmental effects had been better studied. Meanwhile the manufacturers had started to replace phosphate with NTA in a big way. By the end of last year, Procter and Gamble had substituted NTA for 25 per cent of the phosphates used in its two brands "Gain" and "Cheer". The company's advertisements in March 1970 claimed it was using as much NTA as could be supplied. Monsanto, the main manufacturer, said last December it was midway through expansion of one NTA plant and was building another.

What better moment for the Administration to announce the rules of the game were changed? It so happened that on December 19 last year, Senator Muskie was scheduled to hold hearings on the problem of detergents and pollution. On December 17 the detergent manufacturers were summoned to Washington and confronted by Nixon officials with data indicating that NTA could damage the foetuses of pregnant rats and mice. The data, gathered by the National Institute of Environmental Health Sciences, showed that chelate compounds formed between NTA and cadmium (commonly present in water supplies) or methyl mercury were teratogenic to those species. Surgeon General Jesse L. Steinfeld and William D. Ruckelshaus, head of the Environmental Protection Agency, gave the companies until the next morning to decide whether they would withdraw NTA from the market or fight. The companies capitulated. Asked at a press briefing why the government had encouraged industry to use NTA, Ruckelshaus said it was "open to some dispute" whether the government had actually done so, but no evidence of discouragement was produced. It is hard to know which was more irresponsible, the industry's decision to launch into NTA without having conducted a seemingly obvious experiment, or the government's connivance in the manufacturers' action and its failure to ensure they had undertaken the necessary experiments.

The retreating enemy was further harassed a month later by the Federal Trade Commission which proposed in January this year that even detergents which do not contain NTA should bear a public stigma of their environmental obloquy. If the FTC has its way, all advertisements and packaging for detergents containing phosphate will have to carry the following statement: "Warning: each recommended use level of this product contains (amount in grams) of phosphorus, which contributes to water pollution." (The detergent manufacturers argue with some justice that eutrophication is not precisely the same as pollution.)

The proposed label adds, with per-

haps excessive solicitude, "Do not use in excess".

Two other attempts by detergent makers to get out of phosphates have also fallen under the zealous scrutiny of environmentalists and Washington politicians. Enzyme detergents, a business now worth \$25 million a year, first appeared in American supermarkets in 1968. It was only in February this year that the Food and Drug Administration, stirred by the complaints of consumer advocate Ralph Nader to the Federal Trade Commission, set about seeing whether enzyme detergents present a hazard to users. As a result of meetings with the FTC, the FDA has asked the National Academy of Sciences to undertake a six month study of the enzyme question.

Another phosphate substitute that has come more rapidly to grief is the skillfully named Ecolo-G. Launched last July in leafy green packets by the North American Chemical Company, the detergent was soon being distributed in 25,000 stores throughout the United States and promised to gross its manufacturer \$15 million worth of sales within the first year. "Stops pollution, no phosphates!" claimed the slogan on each packet of Ecolo-G. The large detergent manufacturers acidly commented that Ecolo-G and the several other no-phosphate detergents on the market were merely reformulations of old ingredients whose high alkalinity could irritate the housewife's hands. "It's all just sour grapes from the phosphate establishment", Mr Louis D'Almeida, Executive Vice-President of North American Chemical, was quoted as saying last December. Ecolo-G had brought him thousands of thank-you letters from housewives, because "people were looking for something that could help them do their bit for ecology". Ecolo-G seemed off to a more propitious start than a previous North American product, a dishwasher detergent ordered off the market by the FDA in 1969. The colourful company had had another brush with authority in the mid-1960s when a sales company it had hired was accused of having Mafia connexions and threatening arson to potential stockists.

Surprisingly, in view of the thousands of thank-you letters from housewives, FDA officials were less than happy with Ecolo-G and an identical brand known as Bohack No Phosphate. "They're toxic, corrosive to intact skin and produce, on contact, a severe eye irritation," an FDA official said this month after cases of the two detergents had been seized by federal marshals. "They create an open wound on the skin, an actual burn. I've never seen anything like it," he added. North American officials testily replied, "This product is only for clothes and washing machines.

It doesn't matter whether it's toxic or not. What are you going to do, eat it?"

The harmful ingredient of Ecolo-G, according to the tests conducted by the FDA, is sodium metasilicate. Last week the agency announced that Ecolo-G and Bohack No Phosphate would be allowed back on the market provided each packet carries a label warning of its toxicity, together with the encouraging instruction, "If swallowed, give large quantities of water or milk. Follow with citrus juice or dilute vinegar. Call physician immediately."

The detergent story is all good soap

MEETINGS

Post-mortems on AAAS

How can scientific meetings be protected from minority groups determined to disrupt them? The problem has surfaced with particular vehemence at the last two annual meetings of the American Association for the Advancement of Science, at the latter of which the radical group coordinating the disruptions issued the warning "No scientific societies will meet again without our collective voice being heard".

Some suggestions on how to cope with disruptions have been proposed by Joseph F. Coates, a staff associate at the National Science Foundation. The sessions chaired by Coates at the AAAS meeting in Chicago last year were the scene of persistent and violent dissensions, including the widely reported assault on a demonstrator by a professor's wife with a knitting needle. Writing in the March newsletter of the Science and Public Policy Studies Group, Coates says that while a court injunction may be the ultimate defence against disrupters there are several more constructive counter-measures that may obviate so extreme a measure.

One step is for societies to arrange for the participation of dissenters when planning their programmes and to encourage dissident groups to set up their own sessions. Chairmen of sessions "should not resort to trickery, or what can be interpreted as trickery, to discourage or suppress uncongenial discussion". The rules governing changes in the programme and the handling of disruption should be made clear beforehand so as to make clear who is breaking and who is subscribing to them.

Coates comments that general anti-establishment radicals (a class from which he excludes blacks and women) are "usually without analytical, intellectual preparation and generally behave so as to suggest that they are incapable of going beyond the disruption... these zealots are by no means children. Their average age was about 28 at the AAAS meeting. They are well-trained men, or

opera, but is it good government? As with cyclamates and the SST, it is a story of a solution to an environmental problem, doubtless a necessary and in the end correct solution, being achieved by costly and inefficient means. The case is one of environmentalists overstating their arguments, in the belief that this is the only way to get a hearing, while politicians and rival government departments compete for a piece of the action. The decisions made in such an atmosphere often give the impression of being designed more to kill technology than to control it.

in some cases long-term practitioners of the scientific enterprise. The most alarming aspect of their behaviour was... the complete overriding of the wishes of the people and their institutions coupled with baseless, relentless litany of wild charges and an absolute unwillingness to engage in, or to permit, rational discussion."

One point on which both Coates and his disrupters agree is that the latter's tactics, at least in their more extreme form, are counterproductive. SESPA (Scientists and Engineers for Social and Political Action), the group which organized the disruptions at the AAAS meeting, confess in the February issue of their publication *Science For The People* "we learned that moralistic *ad hominem* attacks are self-defeating—we must do our homework and analyse the institutional framework of science...". The SESPA article claims, however, that the intervention at the session on crime, violence and social control chaired by Dr Coates "succeeded in changing the structure and stimulating participation". The chairman was "replaced", the meeting opened up, and "long-constrained ordinary people full of life experience" rose to speak in place of the "usual sterile reading of a paper".

Disruptions at the sessions chaired by Coates were probably the least rational of any at the AAAS meeting, and the manifestations of the same SESPA group at the American Physical Society meeting in New York in February were considerably more restrained. But the more unreasoning forms of student dissent continue. Edwin H. Land, chairman and research director of the Polaroid Corporation, recently cancelled a lecture on colour theory at Harvard University because of threats to break up the meeting, and last week a lecturer at a pro-Administration seminar on the Indo-China war was prevented from speaking at the Sanders Theater, Harvard, by the chanting of a group in the audience.

NEWS AND VIEWS

Controlling Chromosome Loss

IN the past two years the study of human genetics has undergone a remarkable change. Establishing human gene linkage groups and assigning genes to particular chromosomes used exclusively to be a matter of tracing the familial inheritance of a particular trait, an abnormal enzyme or some rare genetic disease for example, and coupling the data obtained with cytogenetic analyses of the individuals concerned. But all that has changed. What used to be a subject curtailed by the inherent restrictions of purely observational science, compounded by the problems of working with people, has now become an experimental science as well. For the development of the technique of inducing cell fusion with inactivated Sendai virus, together with the observation that mouse-human hybrids preferentially shed their human chromosomes, has meant that human genes specifying proteins distinguishable from their murine counterparts can be assigned to particular human chromosomes by analysing the enzymatic and chromosomal constitution of mouse-human hybrids. Leaving aside complications that can arise from processes such as translocation, a mouse-human hybrid cell should only contain a particular human enzyme so long as the hybrid retains the human chromosome that specifies that enzyme. And of course human linkage groups can be established or excluded by experiments of this type. In short, human geneticists now have two quite distinct ways of answering the questions they wish to ask: the classical approach and the experimental approach.

But the use of hybrid cells suffers from restrictions. For one thing, only mouse-human and Chinese hamster-human hybrids preferentially shed their human chromosomes; hybrids involving human cells and the cells of other species are not so obliging and shed chromosomes of both parents in unpredictable patterns. And for another,

many human proteins cannot readily be distinguished from their murine or Chinese hamster counterparts. What somatic cell geneticists need, of course, is a general method which allows them to dictate which parental set of chromosomes shall be shed from any interspecific or intraspecific hybrid. On page 367 of this issue of *Nature*, Pontecorvo describes some preliminary experiments which suggest it may be possible to do just that by irradiating cells before making hybrids with them.

Remembering that some thirty years ago, while working with *Drosophila*, he had solved an analogous problem by X-irradiating cells, Pontecorvo produced hybrids by fusing irradiated Chinese hamster cells with mouse cells. Normally hybrids of these two species of cells do not preferentially shed either set of chromosomes. But hybrids including the irradiated Chinese hamster cells preferentially shed Chinese hamster chromosomes. Apparently the breaks induced by irradiation in the Chinese hamster chromosomes result in their elimination from the hybrids. As an alternative to irradiation, Pontecorvo also allowed Chinese hamster cells to incorporate bromodeoxyuridine into their DNA, then he exposed the cells to visible light, which induces breaks in DNA strands at the sites of the bromodeoxyuridine residues, and then he fused the cells with untreated murine cells. This procedure also resulted in the preferential loss of the Chinese hamster chromosomes from the hybrids. Although preliminary, this result is particularly interesting because it holds out the possibility of selectively eliminating individual chromosomes from hybrids. It might be possible to synchronize pulses of bromodeoxyuridine with the time of duplication of the DNA of a particular chromosome which would, as a result, be selectively sensitized to visible light and subsequently to elimination from a hybrid.

Lunar Origin Theories blow Hot and Cold

THE direct analysis of lunar dust and rocks in the laboratory has failed to resolve conclusively the question of whether the Moon has a hot or cold origin, although the theory which is currently favoured is that the presence of a large amount of crystalline igneous material must certainly imply a history of thermal activity. The samples from Apollo 12, in particular, also show that substantial fractionation of the primary lunar material has taken place, although there is also clear evidence that the surface layer itself is debris resulting from intense meteoritic bombardment, that some craters at least are the result of meteor impacts.

But in the study of lunar magnetism there is less background of speculation and half theory to cloud the interpretation of new results. It was only in 1967, with the flight of Explorer 35 (a Moon orbiting satellite carrying a magnetometer), that direct observations of the magnetic field near the Moon and its interaction with the solar wind were first obtained. Following this success, a magnetometer experiment set up by the crew of Apollo 12 and provided by the same team from the Ames Research

Center in California reported an intense local field of 350 microgauss—this could not represent an overall lunar field like that of the Earth, because such a field would register on the still operational Explorer 35 magnetometer, and is probably caused by a large mass of magnetized rock in the vicinity of the Apollo 12 landing site. This concept of areas of “fossil” magnetization fits well with the discovery of magnetized rocks in the samples returned to Earth, and raises interesting questions concerning the early history of the Moon.

The most exciting results from the Apollo 12 experiment concerned variations in this lunar surface field. Last year, Professor C. P. Sonett presented to the NATO Advanced Study Institute at the University of Newcastle upon Tyne a preliminary interpretation of these variations, which are associated with the variations of the solar wind, and suggested the presence of a stratification in the deep Moon and the existence of a conductive layer at a depth of a few hundred kilometres. Sonett and his colleagues now report in this issue of *Nature* (page 359) a detailed analysis which confirms the existence of a

layer of high conductivity at a depth of ~ 250 km, and also reveals further stratification overlying a uniform core of primaevial matter. The amplification of the lunar magnetic field produced by sudden increases in the planetary magnetic field is the key which has unlocked this secret. If the Moon were a homogeneous sphere of conducting material, electromagnetic induction could be expected to produce a current flowing so that its associated magnetic field would be dipolar, opposing the interplanetary field where this was incident normal to the Moon's surface and doubling the interplanetary field where it was tangential to the Moon's surface.

Surprisingly, however, the amplification of the increasing interplanetary field can be much greater than by a factor of two. The most plausible explanation of this phenomenon is that a dipole field induced in a conducting inner layer is compressed by the solar wind into the less conductive outer shell—although it should be borne in mind that the lunar geometry will not be quite as simple as a series of concentric spherical shells, this explanation is unlikely to be greatly affected by the known amount of anisotropy in the Moon. The conductivity required for the conducting mantle implies a temperature of between 450 and 950°C , depending on composition. So the thermal gradient from the surface to this mantle is about 3 K km^{-1} . Sonett *et al.* believe that the evidence for fractionation found in the available samples is equally well explained by the heating which would occur during the early life of the Moon as a result of the current generated in the conducting layer from a strong interplanetary electric field associated with the rapidly spinning pre-main sequence Sun; evidence for the sort of field required to fit the lunar observations is clearly apparent in the very young T Tauri stars.

There is therefore a very plausible history for the Moon, in which it had a "cold" origin—forming from the accretion of interplanetary dust—and was then subjected to heating of a non-nuclear origin during its life. Locally, there are regions of the mantle which have been heated enough to produce the observed lava flows, but the core itself has never been molten, and, although its temperature today is slowly rising, it is still composed of the original solid matter from which it formed.

EARTH'S CRUST

Hidden Transform Faults

from our Geomagnetism Correspondent

ICELAND is different things to different people, but to Earth scientists it is the only large land mass lying across the mid-Atlantic Ridge system. The Reykjanes Ridge enters Iceland from the south-west and the Jan Mayen Ridge leaves it towards the north-east; but it is what lies between that particularly interests geologists even though, by definition, it is hardly a typical spreading region. Is the ridge beneath Iceland a single continuous strip between the north end of the Reykjanes and the south end of the Jan Mayen? Or is it broken in some way, by transform faulting or otherwise? The Reykjanes volcanic zone in south-west and central Iceland is marked by en echelon faults which are probably the surface effect of deeper transform faulting. Indeed, Ward *et al.* (*J. Geophys. Res.*, **74**, 665; 1969) extended this faulting to the volcanic zone in eastern Iceland which they took as the present crest of the active ridge.

Within this system there are, however, two curious anomalies. For one thing, with both western-central and eastern volcanic areas there are suggestions of two active ridges, and for another there is Snaefellsnes—a volcanic zone, Pleistocene and Holocene, which forms a 120 km long east-west lenticular pile in the extreme west centre of Iceland. In other words, the Snaefellsnes zone runs perpendicular to the central ridge trend and, moreover, produces basalts which are chemically distinct from those produced in the eastern and central volcanic areas.

So why Snaefellsnes? It is hard to be certain, of course; but Sigurdsson (*Earth Planet. Sci. Lett.*, **10**, 129; 1971) has come up with a hypothesis which can explain the matter in general terms if not in detail. He supposes first that during the Pleistocene the eastern volcanic zone, which was the major north-south active zone and stretched the length of Iceland, was joined by a second active zone parallel at about 100 km to the west and again stretching the whole north-south length of Iceland. For a while, from about 1.5 to 0.5 million years ago, both zones lived happily together and spread merrily away. But during the late Pleistocene the northern half of the western-central zone ceased activity whereas the southern half remained active until at least the Holocene. At any rate there came a time when both zones were spreading below latitude 15°N but only the eastern zone was spreading north of this latitude. It is possible, of course, that the total spreading from the two southern zones was no greater than the spreading from

the northern half of the eastern zone; but in so far as the eastern zone appears no wider in the north than in the south this is unlikely. Sigurdsson believes that there really was a differential spreading between north and south and that as a result something had to give.

What gave, of course, was the east-west boundary separating the regions of grossly differing spreading rates—and so Snaefellsnes was born. An east-west transform fault appeared between the two spreading zones and extended westwards through the Snaefellsnes region as a transcurrent fault. In other words, the Snaefellsnes volcanic zone is in theory of a different type from the central rift and transform zones; and this is supported in practice. The heat flow there, for example, is not anomalously high and the alkaline and transitional basalt volcanism produced there is in marked contrast to the tholeiitic volcanism in the other zones. Sigurdsson's hypothesis thus seems to fit the situation rather well; but at the same time there are a few not insignificant problems to overcome and which require more work in the field.

In the meantime, under vastly different circumstances and for rather different reasons, Bacon and Gray (*Earth Planet. Sci. Lett.*, **10**, 101; 1971) also think they have found a transform fault—albeit an old one—this time off Spain. What Bacon and Gray have done is to fill in another section of the almost complete gravity map of the Bay of Biscay. The free air anomalies in the Bay and especially in the newly mapped east are predominantly negative, indicating an area of subsidence and sediment accumulation. The large gravity low off northern Spain, in particular, Bacon and Gray interpret as an ancient fracture zone "along which a pre-Cretaceous west to east movement of Iberia of some hundreds of km occurred after the formation of the Bay".

The rationale behind this supposition is this. There is some evidence to suggest that the Bay of Biscay was formed by the rotation of Iberia about a pole at the western end of the Pyrenees, a phenomenon which is apparently contradicted by the fact that the Pyrenees show no increase in estimated crustal shortening from west to east. Bacon and Gray suggest that this problem may be overcome by assuming that after the rotation about the western Pyrenees pole the Iberian peninsula was left several hundred kilometres to the west of its present position relative to the rest of Europe. A subsequent easterly movement would then have been required.

BUBBLE CHAMBERS

New Ones on the Way

FUTURE bubble chambers could be very much more flexible in use than their predecessors and one of the ultimate goals is to make an almost continuously sensitive bubble chamber which can be counter controlled (in the same way as a spark chamber system) and photographed only when an interaction of particular interest has taken place.

Until a year or so ago the bubble chamber was accepted by elementary particle physicists as a particle detector which, by its nature, could only be expanded every few seconds regardless of the interactions which had taken place inside it. The laborious scanning of the photographs for interesting interactions was a satisfactory procedure as long as interaction cross-sections (probabilities) remained fairly high. But as interactions of lower cross-section are looked for, fewer and fewer of the photographs contain useful material.

The new bubble chambers have come to be known collectively as fast cycling chambers and these divide naturally into those which operate on a variation of the traditional bubble chamber principle (pressure lowering by the rapid release of a piston) and the more esoteric varieties such as sonic and ultrasonic chambers. The rapid cycling chambers broadly include those which can be photographed at rates in excess of 20 per second. (Several existing chambers have, however, been modified so that they can be expanded ten or so times during a typical accelerator pulse (about 1 second), but this is about the limit.) Although a great deal of pioneering work has been done at accelerator laboratories around the world including Dubna (USSR) and the Rutherford High Energy Laboratory (UK), perhaps the greatest interest at the moment centres on a rapid cycling chamber which the Bubble Chamber Development Group at Stanford are building for use in the experimental programmes at the linear accelerator. The diameter of the sensitive volume is about 40 cm, a dramatic improvement on the 5 cm chamber which they successfully operated at 90 Hz in 1969. The expansion system is essentially an electromagnetically driven piston whose natural vibration frequency is 120 Hz—but to ensure proper recombination of the bubbles between cycles, it is only planned to use it at 60 Hz.

It is quite probable therefore that a combined spark chamber-bubble chamber arrangement will soon be in operation at Stanford. In general terms this means that it will be possible to use the hydrogen in a rapid cycling chamber as the target for interactions and for detecting decays and other events of interest which take place very close to

the interaction vertex; these would probably be missed if a separate target was placed in front of a set of sampling detectors such as spark chambers.

In many ways, the sonic and ultrasonic chambers are rather lagging behind the rapid cycling chambers. The principle of operation of both is that the pressure changes necessary for bubble formation and recombination are created by a standing acoustic wave. Because sound waves with frequencies of a few kHz have wavelengths comparable with typical chamber dimensions, the sensitivity to traversing particles varies from one part of the chamber to another and consequently more effort has been directed towards the ultrasonic chambers which operate at frequencies of about 100 kHz (helium) and 450 kHz (hydrogen). In these chambers alternate closely spaced striations are sensitive to the passage of charged particles and resolutions of about 2 mm have been achieved.

But in practice there is an important snag, especially for the hydrogen chambers which are most likely to be of use in experimental particle physics; it turns out that there is a large impedance mismatch between the piezoelectric crystals which generate the ultrasound and the cryogenic liquid, although the problem is not so great for helium as for hydrogen. The situation was highlighted recently at a meeting held on March 18 at the Rutherford Laboratory under the auspices of the Institute of Physics and the Physical Society. In particular, Dr R. C. A. Brown reported the work of the CERN group which has been hampered recently by breakages and over-heating of crystals during attempts to deliver sufficient energy into liquid hydrogen. They have, none the less, observed some tracks in hydrogen ultrasonic chambers but are intent at present on developing coatings for the piezoelectric crystals which would reduce the mismatch.

Slowing Down RNA Polymerase

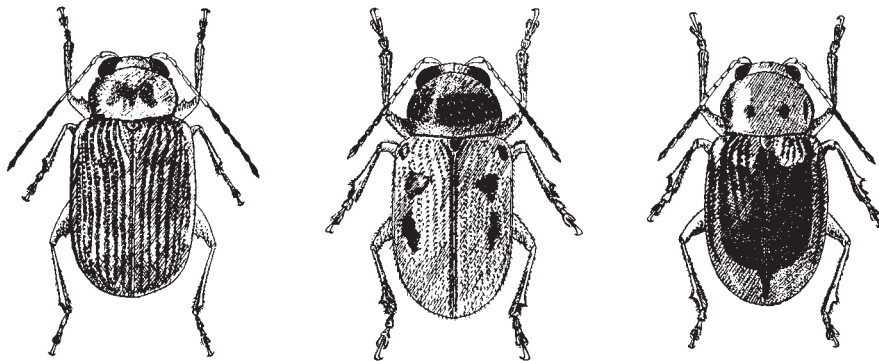
IN recent years antibiotics have provided molecular biologists, not least those investigating RNA synthesis, with more than one lucky break, for compounds which the pharmaceutical industry has isolated because of their bactericidal properties have proved to interfere specifically with such processes as protein synthesis and transcription. Rifampicin, for example, has as its target the act of initiation of transcription; in its presence no new RNA chains are initiated, although those already begun are completed, and the drug has, of course, helped greatly in sorting out precisely how the synthesis of an RNA begins. Streptolydigin, another antibiotic, inhibits RNA polymerase at all stages of transcription and as Cassani *et al.* report in next Wednesday's *Nature New Biology*, it does this by reducing the rate at which RNA chains grow without affecting the fidelity of transcription. As a result, by judiciously manipulating doses of rifampicin and streptolydigin, biologists can control not only the initiation but also the rate of elongation of RNA chains being synthesized by bacterial RNA polymerase.

Binding studies reported by Cassani *et al.* indicate that streptolydigin binds either to RNA polymerase or to the complex of RNA polymerase and its DNA template but not to the DNA template alone. This binding is weak and readily reversed, but when the drug is bound to the enzyme-template complex it freezes the complex and prevents the growth of the nascent RNA chain without causing the enzyme and the RNA product to dissociate from the DNA template. Furthermore, measure-

ments of the binding of the four nucleoside triphosphate precursors to the enzyme-template complex suggest that in the presence of streptolydigin the affinity for CTP is increased slightly whereas that for UTP is decreased. These changes do not, however, greatly affect the fidelity of transcription, for messenger RNAs made, albeit slowly, in the presence of the drug are functional. In short, this antibiotic seems to tighten the interaction between enzyme and template and so reduces the rate at which the enzyme moves along the template.

A drug which is likely to become widely used by biologists probing transcription by mammalian rather than bacterial RNA polymerase is α amanitin. A cyclic peptide produced by the toadstool *Amanita phalloides*, α amanitin specifically inhibits the RNA polymerase found in the nucleoplasm of mammalian cells and can be used to distinguish this enzyme from the RNA polymerase in nucleoli. In *Nature New Biology* next Wednesday, Fiume *et al.* describe a new technique for promoting the uptake of amanitin by liver cells. They have shown that by coupling the β form of amanitin first to albumin and then tagging this complex with fluorescein the toxicity, which reflects uptake, is enhanced some three to four-fold compared to that of amanitin coupled only to albumin. Admittedly the ability of the complexed drug to inhibit RNA polymerase is reduced some five-fold, compared with that of amanitin alone, but when it comes to investigating the effect of drugs inside cells it matters little how potent the drug is at inhibiting a particular enzyme if the drug is not readily taken up by the cells in question.

COLEOPTERA

New Species of *Metachroma*

In a review of beetles in the genus *Metachroma* (family Chrysomelidae) in the western hemisphere, Doris H. Blake describes (*Smithson. Contrib. Zool.*, No. 57; 1970) forty-seven new species, three of which are illustrated here: *M. ignotum*, *M. hirsutum* and *M. bimaginatum*.

POLYPEPTIDES

Progress on Proinsulin

from our Molecular Biology Correspondent

THE relation between insulin and its precursor, proinsulin, is now receiving the kind of minute scrutiny that was lavished on the activation of the pancreatic zymogens—a subject about which enough has surely been written to fill a modest library. Bovine proinsulin is a chain of eighty-one residues, of which thirty are excised when it is activated—a much greater fraction of the chain than in any known zymogen-enzyme conversion. Steiner and his colleagues (*J. Biol. Chem.*, **246**, 1365; 1971) have now established that the conversion involves more than one proteolytic step, but that the major product is a peptide of twenty-six residues (C-peptide), which remains, together with the insulin, in the pancreatic granules.

The proinsulin fraction as isolated contains two intermediates, one cleaved at the junction of the C-peptide and the insulin A-chain, the other at its junction with the B-chain, and in both cases two amino-acids have been lost. The proinsulin chain can thus be represented, reading from the N-terminus, as B-chain, followed by arg-31 and arg-32, which are lost during activation, followed by the 26-residue C-peptide, then by lys-59 and arg-60—again lost on activation—and finally the A-chain. This remains attached to the B-chain by two disulphide bonds, and doubtless non-covalent interactions. Steiner *et al.* give a revised sequence for the C-peptide, and show that it is identical with the product of trypsin-cleavage of proinsulin *in vitro*. The insulin and the C-peptide in the pancreatic granules are recovered in equimolar proportion.

The peptide has a curious composition, with a very high content of proline and of glutamic acid. One supposes that the cleavage *in situ* is effected by trypsin, in just the same way as *in vitro*, and that the ends of the insulin so liberated are trimmed off by carboxypeptidase B.

In a companion paper Oyer *et al.* (*ibid.*, 1375) describe the preparation and

sequencing of the C-peptide from human pancreas. There are not twenty-six but thirty-one residues, and two amino-acids are again lost at either end. A comparison of the sequence with that of the bovine and porcine C-peptides previously determined shows that, making allowance for differences in length, only half the positions are invariant. Because the C-peptide can be presumed to lie on the outside of the molecule, and its removal does not interfere with the packing of side chains in the residual globular insulin molecule, this relatively low degree of conservation, relative to that found in A-

and B-chains, is perhaps not unreasonable.

Earlier work from the same laboratory showed that refolding of fully reduced denatured proinsulin was an efficient process, by contrast with correct association of separated A- and B-chains, which can be achieved only in relatively low yield. Oyer *et al.* suggest that therein may lie one of the advantages of the existence of a precursor. One may note in this connexion the recent work of Varandani and Nafz (*Arch. Biochem.*, **141**, 533; 1970), who studied the refolding of proinsulin from the "scrambled" form, with the six half-cystine residues randomly paired, under the influence of a refolding enzyme, which catalyses disulphide exchange. They found that the native structure of proinsulin was readily restored (as judged by immunological assay), but native insulin could be regenerated in this system only in the presence of C-peptide (four residues of the complete proinsulin sequence being of course missing). The implication is that the C-peptide binds to insulin, rather in the manner of S-protein and S-peptide in subtilisin-cleaved ribonuclease. Such an interaction is not, however, apparent from the work of Steiner and his colleagues, who find that insulin and C-peptide even circulate independently in the serum. Evidently, conditions for the interaction have to be closely defined if the results of Varandani and Nafz are to be taken at face value.

Evidence of Charged Heavy Leptons

NBODY has yet come forward with anything approaching a satisfactory explanation of the existence of the muon and the electron although many attempts have been made to prove that the muon is something more than just a heavy version of the electron. It may, however, be that there are "elementary" particles, intimately associated with these two, which have so far managed to evade detection in particle physics experiments.

The next issue of *Nature Physical Science* includes an article by C. A. Ramm of the European Organization for Nuclear Research (CERN) in which he describes what may be the observation of very short lived particles ($<10^{-12}$ s) related to the muon and electron (all members of the lepton family). Ramm has carried out an analysis of some of the neutrino induced interactions photographed in a heavy liquid bubble chamber at CERN during 1963–64 and 1967; the ones of particular interest are those in which a muon and a γ ray seem to originate from the interaction vertex.

The properties of any group of particles (such as a muon and a γ ray) can be described by its invariant mass which is simply a mathematical combination of the total energy and momentum of the group. If the muon

and γ ray produced in the interactions which Ramm has studied were related (in the sense of being the decay products of a very short lived particle), a peak would be expected in the invariant mass spectrum at a value of the mass corresponding to the mass of the new particle.

In fact, Ramm has found just such a peak in the invariant mass curve at about 430 MeV. If this peak is genuine—and Ramm produces plenty of evidence to suggest that it is—then the existence of a charged lepton whose mass is about four times that of the muon and eight hundred times that of the electron has definitely been established.

Ramm's conclusion supports his previous proposition of the existence of a heavy neutral lepton with about the same mass (*Nature*, **227**, 1323; 1970) and could also tie in with the work of Liberman *et al.* (*Phys. Rev. Lett.*, **22**, 663; 1969) who examined the invariant mass distribution of muons and their γ ray bremsstrahlung but were unable to make a definitive statement in favour of the existence of heavy leptons. What must now be awaited with interest are the results of experiments which have been carried out at the Stanford Linear Accelerator recently and which could greatly clarify the situation.

NUCLEOTIDES

Fluctuating Pools

from our Cell Biology Correspondent

IN January (*Nature*, **229**, 156; 1971) I drew attention to the elegant experiments of Nordenskjöld and his colleagues who, exploiting their enzymatic assay for deoxynucleoside triphosphates, managed to measure the pool sizes of these molecules in mouse embryo cells. Skoog and Nordenskjöld (*Europ. J. Biochem.*, **19**, 81; 1971) have now used their technique to measure changes in the four deoxynucleoside triphosphate pools during the inhibition and restoration of DNA synthesis following the addition and removal of hydroxyurea and cytosine arabinoside.

After inducing DNA synthesis in resting cultures of mouse embryo cells by increasing the concentration of serum in the medium from 0.5 per cent to 100 per cent, they assayed the pool sizes of dATP, dTTP, dGTP and dCTP as the cultures achieved maximal DNA synthesis. At their peaks, the pools of dATP and dTTP are in the range 3 to 4 pmoles per μg of DNA; the pool of dCTP is about 9 pmoles per μg of DNA whereas that of dGTP, the smallest of the four, is only 0.5 pmoles per μg DNA, which is sufficient to support DNA synthesis for only 30 seconds. When hydroxyurea (1–10 mM) is added to these cells, DNA synthesis decreases rapidly and within a few minutes the inhibition is maximal. Measurements of the pool sizes of the four triphosphates during the establishment of this inhibition reveal an almost instantaneous loss of the dGTP pool and a more gradual decrease in the pool of dATP. The pools of the other two triphosphates dCTP and dTTP increase but the turnover of dTTP is reduced. Following removal of the inhibitor the pools are rapidly re-established at their normal levels but restoration of DNA synthesis lags behind. All these changes are consistent with the suggestion that hydroxyurea blocks the activity of ribonucleoside diphosphate reductase, and because of this, and the very small pool of dGTP, the drug causes very rapid inhibition of DNA synthesis.

Cytosine arabinoside inhibits DNA synthesis in mouse embryo cells at much lower concentrations (1–10 μM) than does hydroxyurea, and it causes very different changes in the sizes of the nucleotide pools. In the presence of cytosine arabinoside the pools of dATP, dGTP and dTTP doubled over a period of half an hour and continued to increase thereafter, albeit at a slower rate. By contrast, the pool of dCTP shrinks during the first hour and only slowly recovers its normal level. The dTTP pool turns over more slowly in cells

treated with cytosine arabinoside than in controls (half-lives 13 min and 4 min respectively), but the pool is two to three times larger in the treated cells. Removal of the drug is rapidly followed by a restoration of the normal pool sizes and subsequently DNA synthesis restarts.

These findings rule out the idea that cytosine arabinoside blocks ribonucleoside diphosphate reductase activity; so how does it inhibit DNA synthesis? Cytosine arabinoside is triphosphorylated in mouse embryo cells and the parallels between the accumulation and loss of aCTP and the inhibition and restoration of DNA synthesis observed by Skoog and Nordenskjöld strongly support the suggestion, first muted by Furth and Cohen in 1967, that aCTP, and therefore cytosine arabinoside, inhibits DNA synthesis by interfering with polymerization. Be that as it may, thanks to the valuable work of Nordenskjöld and his colleagues there is now a much clearer picture of precisely what happens inside cells when they are exposed to these inhibitors.

ORIENTATION

Jumping Spider

from our Animal Behaviour Correspondent

IF a jumping spider (Salticidae) detects a moving object with its lateral eyes, it often turns so that the object can be identified by the principal eyes situated at the front of the head. Depending on the nature of the object, prey catching, mating or escape may then follow. The turn made by the spider is very accurate—it turns through just the right angle to bring the object into the field of the principal eyes.

How is this accuracy achieved? One possible mechanism is by visual feedback resulting from the spider's own movement, but M. F. Land (*J. Exp. Biol.*, **54**, 119; 1971) has shown that such a "closed-loop" system cannot be controlling the turns, because the spider turns correctly even when denied such feedback. Land anchored jumping spiders with a piece of card glued onto the back and gave them light card-

Geometries of Molecular Oxygen Complexes

IN next Monday's *Nature Physical Science*, Mingos presents a general treatment of factors affecting the geometry of dioxygen (molecular oxygen) complexes which might aid in resolving the problem of the geometry of oxygen binding in oxy-haemoglobin and oxy-myoglobin. The basis of the argument is an approximate form of the second-order Jahn-Teller effect. Whereas the first-order Jahn-Teller effect deals with distortions of geometry arising from degenerate electronic states, the second order effect is concerned with states which are merely close to each other (in the approximation used only the ground and first excited states are taken into consideration).

The effect provides a symmetry rule for predicting the stable shapes of molecules (R. G. Pearson, *J. Amer. Chem. Soc.*, **91**, 4947; 1969) and suggests that, in the formation of complexes between Lewis acids and bases, it is only when the donor and acceptor orbitals do not have the same symmetry that distortion leading to a new equilibrium configuration of the complex will be allowed. In the particular case of dioxygen complexes, the distortion which would allow transformation of the π -bonded geometry

$\text{M} \cdots \begin{array}{c} \circ \\ | \\ \circ \end{array}$ (which is energetically preferred if dioxygen acts as a Lewis base) into the

non-linear configuration $\text{M} - \begin{array}{c} \circ \\ \diagup \quad \diagdown \\ \circ \end{array}$ is not expected. Peroxo-complexes and superoxo-complexes may be considered as Lewis acid complexes of dioxygen and will only have a non-linear

geometry if the donating metal orbital is a σ -type orbital. The results are in agreement with observation for a range of mono and bi-nuclear dioxygen, superoxo-metal and peroxo-metal complexes.

For oxy-haemoglobin and oxy-myoglobin the treatment supports the π -

bonded (Griffith) structures— $\text{Fe} \cdots \begin{array}{c} \circ \\ | \\ \circ \end{array}$.

The alternative formulation of these species as superoxo-complexes would not suggest distortion because the necessary electron transfer from metal to ligand orbitals does not involve the metal σ -orbitals. This result must, at present, be regarded with caution for two reasons: first, the treatment is based on an approximation, which has been widely successful but which Pearson considers can only be justified if it works in the application at hand; second, the argument takes no account of non-bonded repulsions between the oxygen atoms and the pyrrole nitrogen atoms of the porphyrin ring. Hoard (in *Structural Chemistry and Molecular Biology* (edit. by A. Rich and N. Davidson), W. H. Freeman, San Francisco, 573; 1968) has argued forcefully that the π -bonded geometry would involve considerable steric strain in haem-oxygen complexes which could not readily be relieved. Mingos suggests that molybdenum (VI) and titanium (IV) π -peroxo complexes, perhaps with constrained ligands, would provide useful model systems for studying the influence of steric effects on metal-dioxygen complex geometries.

board rings which they held in their feet and manipulated as if they were running along a twig. Moving targets were used to elicit turns and caused the spider to rotate its ring away from the object. In this way the spider performed the motor patterns of turning but received no visual feedback because the image of the target did not move across the retinae. The angle through which the spider turned its ring was measured and was found to correspond to the angle between target and the axis of the spider's body. The spiders can therefore orient accurately whether or not they receive visual feedback. This means that the angle to be turned through is calculated accurately before the turn is initiated—a remarkable and still poorly understood feat of calibration.

SOLIDS

New Superlattices

from our Materials Science Correspondent

THE term superlattice was coined by an X-ray metallographer many years ago to describe a crystalline solid solution in which, after appropriate heat treatment, solvent and solute atoms are deployed in an ordered manner. In strict semantic terms, the word only makes sense for alloys such as the Cu/Pd series in which "long-period superlattices" occur: the crystal pattern has a structural discontinuity every l unit cells in one particular direction, where l is a small integer, typically 5. Such a superlattice can be described as one-dimensional. Only recently has the work of Sato and Toth at the Ford Scientific Laboratories provided an understanding, in terms of electronic band theory, for the existence of such superlattices.

Work in several unconnected fields of solid state science has now established that long-period superlattices in alloys are not the only exemplars of the species. In recent months, new forms of one-dimensional and three-dimensional superlattices have been described, and a two-dimensional form was predicted and subsequently observed some years ago.

The new one-dimensional superlattice is a man-made semiconductor structure: a silicon monocrystal slice is grown from the vapour phase in an atmosphere which is rapidly and regularly alternated between phosphine-rich and arsine-rich compositions. The slice grows with plane differentially doped layers at a regular spacing, typically about 150 Å (A. E. Blakeslee and C. F. Aliotta, *IBM J. Res. Dev.*, **14**, 686; 1970). Their work was stimulated by a remarkable theoretical paper by L. Esaki and R. Tsu published a few months earlier

(*IBM J. Res. Dev.*, **14**, 61; 1970). Esaki and Tsu worked out in some detail the band theory and electron transport properties of one-dimensional semiconductor superlattices. In effect, such materials contain mini Brillouin zones bounded by small energy gaps, all interpolated inside the principal Brillouin zone, and at the same time they embody periodic fluctuations of the main energy gap. The effective electron mass becomes strongly dependent on the direction of motion of the electron, and in the author's words, this "leads to virtually a two-dimensional electron gas system".

An important theoretical consequence of this feature is that the drift velocity along the normal to the superlattice layers passes through a maximum as a function of applied electric field, and so at sufficiently high fields (not enough, however, to cause tunnelling and avalanching) the system has a negative differential conductance. In effect, this happens when the electronic mean free path sufficiently exceeds the superlattice period. Such systems, in which the host crystal and the scale, amplitude and nature of the dopant superlattice are independently disposable variables, should give rise to a novel class of devices and open new areas in semiconductor physics, a field which has lost its first fine rapture. The preliminary experimental work by Blakeslee and Aliotta has now shown that semiconductor superlattices can readily be made with the requisite period and amplitude and with a large number of layers, and so it seems that the new field is wide open.

The new three-dimensional superlattice is a regular array of voids in irradiated molybdenum, recently described by J. H. Evans (*Nature*, **229**, 403; 1971). Voids are small cavities in a crystalline lattice produced by neutron or particle bombardment. In molybdenum bombarded with 2 MeV nitrogen, electron-microscopy showed that the voids were arranged on a regular body-centred cubic lattice aping that of the host metal but with a repeat distance around fifty times larger (220 ± 10 Å). This remarkable observation has now been explained by R. Bullough and K. Malen at a recent conference on voids at the University of Reading. They showed that superlattice formation by voids depends on elastic interactions between the voids, which in turn relies on the anisotropic elastic properties of the molybdenum crystal lattice; the theory is able to interpret the scale of the observed periodicity. Presumably the voids migrate by the well-attested process of self-diffusion along their surfaces until the optimum periodicity is established, but this has not been examined. The superlattice contains some vacancies and disloca-

tions, though so far these have not been studied in detail. The nomenclature of a vacancy in a void lattice would seem to offer an intriguing exercise in double negatives.

The dimensional catalogue concludes with the two-dimensional flux-line lattices in type 2 superconductors, predicted in 1957 by Abrikosov and directly observed during the past four years. The lattice consists of normally conducting filaments carrying a magnetic field, and over a range of temperatures below the upper critical field these flux-lines exist in equilibrium and arrange themselves in a two-dimensional lattice with either a triangular or a square unit cell. The important feature of the lattice (which typically has a repeat distance of about 500 Å) is that, in a hysteresis-free type 2 superconductor, the entire flux-line lattice is unstable the moment a finite current flows through a superconductor. The critical field in this temperature range is thus zero unless the lattice can be anchored in some way to the underlying crystal lattice.

A great deal of metallurgical research is currently being devoted to studying anchoring to dislocations and to second-phase particles. Until recently, such studies had to depend entirely on indirect deductions based on measurements of critical currents and fields, but a technique invented by Träuble and Essmann in 1966 has made it possible to observe the flux-line lattice directly. Colloidal particles of iron are allowed to deposit on a superconductor surface, decorate the exits of flux-lines and become fixed in position, so that replicas can later be taken and examined by electron microscopy. A. Seeger has recently published a survey of superconductivity and physical metallurgy (*Metallurgical Trans.*, **1**, 2987; 1970), in which much of the active recent research in this field is summarized and its strategy explained. It seems that flux-line lattices themselves contain all the standard defects—grain boundaries, vacancies, edge dislocations and even disclinations (a species of defect which, though specified by theorists such as Nabarro, has not been observed in crystal lattices). After illustrating all these defects in the flux-line lattice, Seeger goes on to discuss the physical nature of their interaction with defects in the crystal lattice itself and the consequence of such interaction for superconducting behaviour. This article is a particularly readable introduction to a subject which bristles with conceptual difficulties.

It now only remains to seek connexions between these different kinds of macroscopic superlattices. Perhaps void lattices can be created in a periodically doped superconductor and unprecedentedly large critical fields thus obtained?

A Century of Mathematical Reform

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The Mathematical Association celebrates its centenary this year with a conference to be held at University College, London, on April 14-16. The programme will include lectures and discussions, and the library of University College will display some of its rare and ancient mathematical books. In this article, a former President of the Association outlines its beginnings and traces its subsequent development.

THE issue of *Nature* for May 26, 1870, contained a short letter suggesting the formation of an anti-Euclid society. The writer was Rawdon Levett, mathematics master at King Edward's School, Birmingham; his iconoclastic proposal represented the opinion of a growing number of teachers, that the current practice of forcing school children to learn Euclid's text by rote, with or without any understanding of its content, was simply barbarous. Levett's idea was taken up by other reformers; among them was J. M. Wilson, later a Canon of Worcester but at this time a mathematics master at Rugby, where the headmaster, F. Temple, afterwards Archbishop of Canterbury, was pressing for a new look in school geometry. Another supporter was Professor T. Archer Hirst, then at University College, London, but shortly to become the first Director of Studies at the newly established Royal Naval College, Greenwich. An Association for the Reform of Geometrical Teaching was founded and held its first formal meeting of some two dozen members on January 17, 1871, at University College, with Hirst in the chair and Levett as secretary. Almost immediately, "improvement" was substituted for "reform" in the title, perhaps lest the members should be classed among the wild radicals of the time. The change did little to propitiate opponents, particularly well entrenched at Oxford and Cambridge; the Oxford don, C. L. Dodgson, better known as Lewis Carroll, in his satirical play, "Euclid and his modern rivals", was wittier but no less reactionary than many other school and university teachers. Not until about 1900 did the two older universities relax their euclidean requirements for entrance, after a struggle in which A. R. Forsyth was a leader of the reformers.

In its early years, the AIGT naturally devoted its principal energies to the task of replacing Euclid by an arrangement and development of geometrical ideas more suitable to the needs of school children. But as its numbers grew, its aims widened to include all mathematics in the middle and upper school range; this expansion was recognized by a change, in 1897, to the present name of the Mathematical Association. Moreover, in response to a suggestion by some members that the Association's aims and activities should be given more publicity, a journal was founded, the *Mathematical Gazette*, the first number appearing in April 1894: number 392, to appear in April 1971, will be a special issue to celebrate the Association's centenary.

The engineer, John Perry, preaching to the British Association in 1901, denounced the divorce of school and university mathematics from the mathematics required by the engineer and technologist. Forsyth headed a committee of investigation, while as President of the Mathematical Association in 1903-4 he encouraged a parallel development urged by some influential schoolmaster members of the Association, to systematize and intensify the Association's efforts to improve and publicize methods of mathematical teaching. A standing Teaching Committee was charged with the duty of constantly reviewing the progress of methods of teaching and of providing reports on their investigations. Some small reports appeared before 1914, but the full force of this policy began to be felt in 1923 with the *Report on the Teaching of Geometry in Schools*. A powerful team, led by T. P. (later Sir Percy) Nunn and E. H. Neville, produced a scholarly but practical document which initiated a revolution in geometrical teaching in this country. Since then, almost every branch of school mathematics has come under the scrutiny of the Teaching Committee and its reports have been accepted here and overseas as constructive and authoritative. A feature of recent work has been an extension to the field of the primary school, culminating in the 1970 report on primary mathematics, so that now the whole range of mathematical teaching in the schools is covered. Recent developments, such as the drastic revision of the school syllabus, and preparations for metrication, have been studied with care. A new journal is about to be published; it will concentrate chiefly on the more elementary stages of mathematical teaching, while the *Gazette* will then deal mostly with the more advanced levels.

The policy of establishing local branches, each with its own programme of meetings and discussions, began before 1914 and developed rapidly between the wars; there are now more than 30 such branches in this country, as well as several in other parts of the Commonwealth. The Association's library, built up almost wholly by gifts and exchanges, contains much material related to mathematical education, and is particularly rich in its collection of periodicals concerned with the history and teaching of mathematics. The library is housed in the University of Leicester and an efficient postal service to members is supplied.

A post-war development of importance has been the establishment of the Association's Diploma in Mathematics, giving a qualification now officially recognized for a "merit" allowance. The standard is approximately that of one year of full-time study beyond A-level; the curriculum contains not only the standard branches of elementary mathematics, as at present interpreted, but also statistics, and the history and development of mathematical ideas. Teachers in service have found that through the Diploma they can add considerably to their teaching range, and thus this activity has done something to mitigate the present shortage of mathematics teachers.

The Association's urgent need at the moment is for a home of its own, without which it can no longer adequately cope with its many and growing activities. Preliminary plans have made good progress, so that all that is now required is a sufficient sum of money; an appeal will be launched as part of the centenary celebrations. If the response is generous, the Association can embark on its second hundred years with confidence and enthusiasm.

Problems for the Coal Industry

RICHARD BAILEY

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The price of coal in Britain is increasing because supplies are inadequate. Should an annual price review be instituted?

THIS year for the first time for fifteen years the National Coal Board can sell every ton of coal mined. Problems of costs and prices remain, however, as the report of the National Board for Prices and Incomes has shown¹. How has this situation arisen?

The short answer is that the promised emergence in Britain of a four-fuel economy has not worked out as expected. The White Paper on Fuel Policy², published in 1967, stated that "on any tenable view on the long term pattern of energy supplies and costs, the demand for coal will continue to decline. This is not the result of Government policy, it reflects a continuing trend in consumer preference". Britain's energy needs are still met by coal and oil with natural gas and nuclear energy making only a small contribution. With natural gas there have been difficulties in the massive conversion programme which has been undertaken.

The result of these changes is a seller's market for coal. Beside the Government's objective of checking price increases, this creates various problems. Broadly, there are three ways of dealing with a seller's market. The first would be to produce more coal at home so that supplies were adequate to meet demand. The National Coal Board is trying to do this by keeping open pits previously considered uneconomic, and by using technically advanced mining methods.

The second possibility would be to bring in supplies from abroad. This would mean dropping the ban on imports and allowing coal to come in from the United States, Poland, the European Economic Community and anywhere else where supplies were available.

The third solution would be to increase supplies of other fuels. In the long term the solution to the energy problem will clearly come from developments of this kind. The National Coal Board supplies about 76 million tons of coal, about half its annual production, to the Central Electricity Generating Board and a further 27 million tons to coke ovens, with 50 million tons equally divided between industrial and domestic consumers, including some export tonnage. The biggest savings could obviously be made by introducing other fuels into the power stations and the steel industry. Conversion to oil is the best option for the power stations but this takes time and involves additional capital expenditure. The use of natural gas for crude heating purposes instead of the premium uses for which it is best suited is wasteful and can only be regarded as an emergency measure. The nuclear power station programme cannot be expanded rapidly, and in any case the serious technical problems which have emerged recently are a sufficient reason for proceeding with caution. The steel industry has so far not perfected a process for the reduction of iron ore without coke.

The sometimes conflicting economic, commercial, technical and political objectives of fuel policy must be reconciled within the context of the present fuel situation. The Prices and Incomes Board review of coal prices singled out the failure to maintain rising productivity as the chief reason why the National Coal Board has had to increase prices twice in the past year and wishes to do so again. This ignores the

impact of inflation, which has hit the coal industry through its purchases of materials and equipment, and high wages. The board's chief proposal for the solution of the problem is to introduce changes in the top management structure in the industry in order to strengthen the amount of control exercised from the centre over operations in the field. In other words, the board believes that the commercial problem can be solved by improved operating efficiency, forecasting and planning by managerial changes. It is at least questionable whether closer day to day control from the centre in an industry as large as coal would have positive results, and would not instead lead to a stultification of local initiative. At any rate the National Coal Board considers that its best chances of success lie in giving effective powers and responsibility to its seventeen area directors, subject to overall policy control and accountability.

In deciding what to do it is important to realize that the present strong market for coal could last for the next four or five years, and that the strength of the coking coal market will stretch beyond that. One question which should be asked is whether the price of coal could in this situation be determined by the market. This would, however, mean that rising costs incurred would be reflected in higher prices. In a free market situation, which would involve the removal of protective arrangements, prices would rise to a level which consumers were prepared to pay. In other words a free market would not only determine prices but also the size of the coal industry. With an industry as complicated as coal there are many factors to be taken into consideration before taking so radical a step as this. Any contraction of the industry would have to continue in a way that would safeguard the position of the workers concerned. Clearly the emphasis in such a situation should be on marketing, and the problem should be considered in commercial terms. While a free market system might be going too far a modification of this could provide a solution to the problem. Coal prices affect industrial costs generally and particularly those of the steel and engineering industries. Instead of the present arrangement under which the National Coal Board has to come forward and ask for a price increase whenever conditions change, an annual confrontation with its customers could provide the means of securing greater stability. An annual price review in which the National Coal Board discussed problems of costs, prices, materials and supplies with its principal customers would produce a situation in which the known factors were discounted and the number of unknown ones considerably reduced.

Such an approach would make it possible for the National Coal Board to operate within the commercial realities of the situation. Questions of conversion of power stations to oil would be discussed as factors affecting the total supply position rather than as an attack on the future of the industry. If the nationalized industries which supply products and services used by industry and the general public are to operate effectively they must be able to adapt to changing economic circumstances without being the focus of political attention. If the particular kind of mixed economy which has developed in Britain in the past twenty-five years is to operate effectively, the similarities rather than the differences between public and private sector industries must be emphasized.

Any move towards an application of market principles to the nationalized industries is likely to be a step in the right direction.

¹ *Coal Prices*, Report No. 153, Command 4455 (HMSO, 1970).

² Command 3438 (HMSO, 1967).

Lunar Electrical Conductivity Profile

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Measurements of electrical conductivity profile provide information about the mantle-core stratification, near surface thermal gradient, heat flux and composition of the Moon.

WE report here the first measurements of the bulk electrical conductivity profile of the Moon. These results confirm the strong electrical induction which occurs when the Moon is affected by the interplanetary magnetic field¹⁻⁴. Here we show convincing evidence, based on the harmonic response, for a layer at a depth of about 250 km which has a spectacular and anomalously high value of electrical conductivity. We suggest that this region is a lunar mantle which is divisible into an upper and lower part overlaying what we believe is a relatively homogeneous core of more primitive matter.

Electrical Conductivity Profile

The values of conductivity are based on an iterative least squares fit to an inversion; the method seeks a best fit electrical conductivity profile to an empirical transfer function $A_i(f)$, which is defined by

$$h_{2i}(f) + h_{1i}(f) = A_i(f)h_{1i}(f)$$

where $h_{2i}(f)$ and $h_{1i}(f)$ are the Fourier transformed time series of the magnetic field induced in the Moon at the lunar surface, and the free stream interplanetary magnetic field, respectively. The parameter f is the frequency and the subscript i is x , y or z , corresponding to a coordinate system with x along the unit normal outwards from the lunar surface, y easterly, and z northerly at the site of ALSEP and the lunar surface magnetometer (LSM)⁴. The LSM measures the sum $h_{2i}(f) + h_{1i}(f)$, and the free stream magnetic field, $h_{1i}(f)$, is

measured by the Ames magnetometer on the lunar orbiting satellite, Explorer 35 (refs. 5 and 6).

Using values of $h_{2i}(f)$ and $h_{1i}(f)$ determined from the two magnetometers, A_x , A_y and A_z have been determined from fourteen spectral swaths lasting 1 or 2 h. Because $A_x \sim 1$, the solar wind confining layer ahead of the Moon is thin compared with the depth at which significant induction currents flow. Fig. 1 shows experimental data points for both A_x and a composite, $\bar{A} = [0.5(A_y^2 + A_z^2)]^{1/2}$. The error bars represent standard deviations determined from the fourteen individual spectra. The introduction of $\bar{A}$ enables us to use both A_y and A_z data to improve the statistics. At the higher frequencies $A_y \sim A_z$, whereas at the lowest frequencies $A_z > A_y$. At the lower frequencies a mixture of transverse electric (TE) and transverse magnetic (TM) modes may be present. Theory predicts that the TM mode would increase A_z relative to A_y , in agreement with the observations. The inference is that TM excitation cannot be ignored at a low frequency and must be considered in a more complete analysis. Alternative explanations for the difference in the response between A_y and A_z exist, but usually involve a fundamental electrical asymmetry in the Moon and there is no compelling reason for such a radical assumption.

The preliminary amplification data (transfer function) shown in Fig. 1 are subject to contamination at the higher frequencies from background tones originating aboard Explorer 35. Such tones are occasionally apparent at a very low level in Explorer 35 data. The addition to spectral power at certain frequencies in these data would decrease $\bar{A}$ as is seen at $f=0.03$ Hz in Fig. 1. An alternative explanation for such decreases is that excitation of the Moon in higher order modes is significant. But such an effect places important constraints on the geometry of the sea of plasma waves in the solar wind which drives the Moon over the long period of time represented by the total number of spectra.

The computed values of $\bar{A}$ shown in Fig. 1 are determined by the iterative procedure discussed later. This is carried out using eight frequencies which are listed in the caption to Fig. 1, together with a TE mode theory for a spherically symmetric

Moon. The restriction on the number of frequencies used is a result of limited computer time. We think the differences between the calculated $\bar{A}$ and the empirical transfer function are partially attributable to the various complications of the excitation process together with the possible spacecraft tones, as well as the restrictions on computer time. Because the pressure of the induced magnetic field is significant we have tested those data for field line confinement at variable Sun angles; the data suggest no loss of field lines as the Sun angle varies, and therefore no correction is included for such an effect.

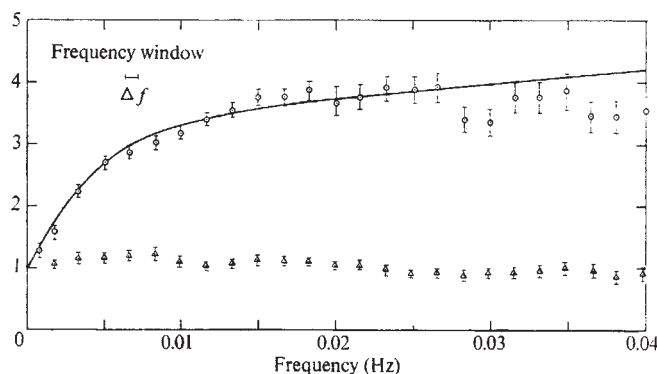


Fig. 1 The r.m.s. tangential lunar transfer function, $\bar{A} = [0.5(A_y^2 + A_z^2)]^{1/2}$ versus frequency; the transfer function for the induction in the direction normal to the surface of the Moon, locally at the LSM site, is shown at the bottom of the figure, and agrees with the expected value of unity. The error bars are the one standard deviation limits determined from the means of 14 data spectra. The frequency windows are defined by the lags in the autocorrelation calculation. The solid line is the value of amplification calculated from the conductivity profile whose corresponding amplification is fitted to experimental values at frequencies of 0.83, 1.7, 5, 12, 17, 22, 25, and 35 mHz. —, Computed $\bar{A}$ (tangential); ○, empirical $\bar{A}$ (tangential); △, empirical A_z (normal).

Fig. 2 shows the conductivity profiles, derived by the iterative least squares inversion, for A_y , A_z and $\bar{A}$. The iterative procedure determines the conductivities at the radial locations $R = 800, 1,200, 1,400, 1,450, 1,490, 1,510, 1,550$ and $1,740$ km. For $R < 800$ km, the conductivity σ is set to the value at $R = 800$ km. Elsewhere a linear interpolation of $\ln \sigma$ is used. The prominent peak, which is relatively independent of the particular A chosen for the calculation, is centred at about $R = 1,500$ km where the conductivity is nearly 10^{-2} mhos m^{-1} . The inner minimum lies at about $R = 1,400$ km and the conductivity seems to rise at a greater depth. Gross bounds on the conductivity profile are shown in the inset; these are determined from the 1 standard deviation limits of the various A s, but do not themselves represent 1 standard deviation limits on the conductivity profile.

As we have noted, the large spike in conductivity is an invariant characteristic of the inversion of A_y , A_z and $\bar{A}$. Deeper in the Moon, however, where the conductivity function seems to rise, the sensitivity of σ to small changes in A is increased, because large variations in core conductivity have a very small effect on the calculation of A . The sensitivity becomes extreme at the lowest frequencies and our results for the conductivity at depth are tentative. The transformation from σ to temperature is logarithmic and will decrease the ultimate error in interpretation. The assessment of conductivity using the limiting values of A_y and A_z should be regarded as a qualitative estimate of the overall probable errors.

Computer Calculation

Each computer calculation started with a constant conductivity of 10^{-4} mhos m^{-1} and permitted the computer to converge towards a solution. In every case, including the extrema,

initial convergence was rapid and the conductivity profile always had the prominent peak. Later convergence was slower; generally we used only five iterations, but errors were tabulated and were always reasonable. The large conductivity spike can be explained as follows. For $A \sim 4$ at high frequencies, geometrical considerations require the skin depth barrier to be near $R = 1,500$ km. Concurrent with the high frequency limitation, A must drop to near unity at the lower frequencies. If it were assumed that the electrical conductivity were monotonic, so that the interior conductivity were uniformly high, then the low frequency amplification would greatly exceed the observed values. Calculations not reported here have shown that the essential nature of the spike is independent of the choice of radial values used in the iterative procedure. For the profiles reported here, several values of R were chosen around $R = 1,500$ km in order better to define the profile.

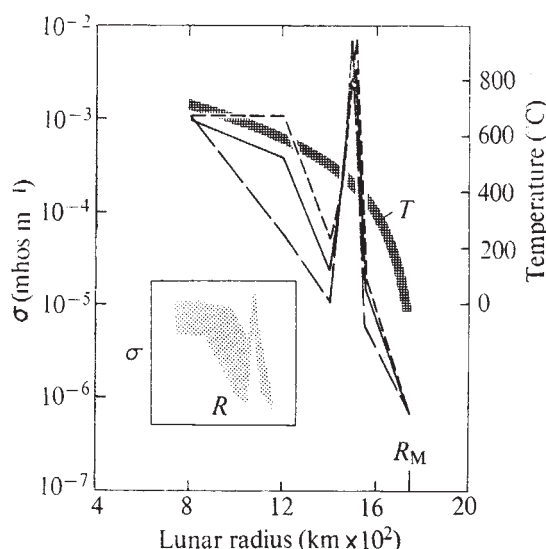


Fig. 2 Lunar bulk electrical conductivity profiles determined from the individual transfer functions A_y (—), A_z (---), and $\bar{A}$ (- · -). The prominent rise of σ from the surface inwards to $R = 1,500$ km is apparent in all three cases as well as the subsequent decrease inwards to $R = 1,400$ km followed by a more gradual rise. The calculated differences for the deeper conductivities are associated with the lower frequency components of the data spectra and may be caused by mixing of the transverse electric (TE) and transverse magnetic (TM) modes. A tentative version of a lunar thermal profile is shown as the grey overlay, with temperatures on the right hand margin. This profile is a fit of conductivities to a Nagata basalt in the mantle, an England olivine in the core, and the known subsurface temperature of -30° C. The inset shows extrema of the possible values of the σ s using the one standard deviation limits of the A s to calculate conductivities. These limits are probably more extreme than the one standard deviation limits of the conductivity profile.

When considering the overall behaviour of the conductivity profile, it is immediately obvious that the anomalous nature of the profile cannot be explained solely on the basis of a uniform material and a well behaved thermal profile for the Moon. In the region from the surface to a radius of $1,500$ km, where the conductivity attains its maximum value, the rise of conductivity with depth is a reasonable consequence of the increase of temperature with depth. But the precipitous decrease of conductivity by nearly 3 orders in the next 100 km is not attributable to temperature because the conductivity gradient has the wrong algebraic sign. There must be either a compositional change, a phase change or a combination of the two. Thus the outer part of the Moon (to a depth of about 350 km) is stratified into at least two principal shells. There is a further division below the anomalous layer of decreasing conductivity where the conductivity again rises

uniformly with depth as a consequence of the temperature rise. A tentative model for stratification of the Moon, limited by the present poor spatial resolution of the analysis, indicates a core of radius 1,400 km overlain by a mantle of anomalous conductivity, with the latter further divisible into an upper and lower part.

Composition of Lunar Mantle

We now inquire into the thermal state of the lunar mantle. The electrical conductivity functions for the Apollo basalts (ref. 7 and unpublished work of Schwerer *et al.*) represent the most conducting rocky matter known, but olivine (dunite) or olivine-peridotite⁸ represents the least conducting geological material. With these extreme functions, the peak conductivity values in the lunar mantle correspond to temperatures of about 350° C and 950° C, respectively. Because the temperature just under the lunar surface, where the solar insulation is well damped, is -30° C, the thermal gradients in the outer mantle are about 2 K/km and 4 K/km, respectively. The precipitous decrease in electrical conductivity of nearly 10^3 as the depth increases from 250 km to 350 km takes place in a temperature rise of at least 100 degrees. If the olivine hypothesis is adopted for the mantle conductivity peak, we conclude that at the bottom of the lower mantle, where the conductivity seems again to rise, the material must be several orders of magnitude less conducting than dunite. Such material is not known on Earth, because of the usual problem of Fe^{2+} and Fe^{3+} substitutional and interstitial additions to olivine.

Lunar Stratification

These conclusions suggest that the Moon is radially stratified, with a core of radius 1,400 km, in composition representative of an ultramafic substrate such as an olivine, or olivine-pyroxene; a lower mantle composed of a material transitional between the substrate and the upper mantle and ranging in radius from 1,400–1,500 km, followed by an upper mantle with the electrical properties of an Apollo basalt. The latter seems to extend upwards almost to the surface; the present upper frequency resolution is insufficient for accurate assessment, and the evidence suggests an anorthositic crust to complete the spectrum of layers⁹. If the core is assumed to possess a conductivity-temperature relationship characteristic of poorly conducting matter, the calculated temperature at $R=800$ km is in the range of 750°–800° C, decreasing to about 550° C at $R=1,400$ km where there is a local minimum in electrical conductivity. A calculation based on more conducting matter would depress the computed temperatures. A combination of an olivine-like core and basalt-like mantle agrees simultaneously with both the thermal and conductivity profiles.

The thermal gradient permits an approximate determination of the heat flux from the Moon. Silicate material has thermal conductivity, K , of $0.008 \text{ calories cm}^{-1} \text{ s}^{-1} \text{ deg}^{-1}$ although it is known that K will decrease with increasing temperature^{10,11}. Estimates show that this effect will not introduce a substantial error in the estimate of $\dot{H}$, the heat flux. Our estimate of the value of $\dot{H}$ is $1.6 \times 10^{-7} \text{ calories cm}^{-2} \text{ s}^{-1}$ using the "basalt" composition for the mantle; olivine would double this estimate. The former value is approximately one eighth that of the Pre-Cambrian regions of Earth¹². For a Moon scaled Earth, the flux would be twice that actually measured (basalt) and approximately equal (olivine).

The volume of the mantle comprises about half of the total volume of the Moon. To fill it with a basaltic extract from below would have required substantial core melting, and this amount of basalt is in conflict with geochemical constraints. Further, the melting temperature increases with depth, ranging from 1,100–1,600° C or higher at the centre of the Moon depending on the assumed composition. We would also have to explain the present apparent upper bound of 800° C, well below most solids, although there is controversy about

convection. Because thermal conductivity decreases with temperature^{10,11} it seems unlikely that the heat loss could be explained in that way. Lastly, the differences in the upper and lower parts of the mantle seem to preclude any common filling of these regions by a melt from below.

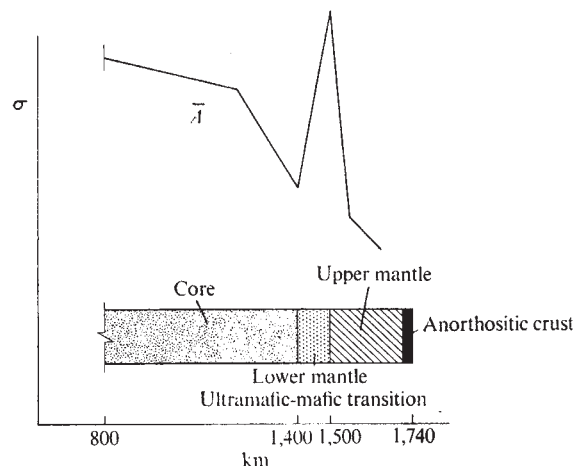


Fig. 3 Model Moon based on the magnetometer data, the derived conductivity profiles, and the consistent fit of σ to rocky material. The anorthositic crust is taken from Wood *et al.*⁹; it is not in the analysis given here.

The alternative explanation for the mantle formation implies an *in situ* thermodynamic regime decoupled from the core. The more refractory materials would have crystallized out and formed the deeper part of the mantle, yielding the gradation in conductivity. The upper mantle would be composed partly of basalt-like substance accounting for the apparent dependence of the upper mantle conductivity simply on temperature. The actual basalt thickness cannot be assayed yet because of limited spatial resolution. Geochemical limits suggest at most 10%–15% by volume, suggesting a layer thinner than so far resolvable. The thermal energy for the fractionation would come from accretional sources, possibly together with radioactives, depending on the exact details of the accretional profile and time scale (ref. 13 and unpublished results of J. A. Wood). If we assume that the initial accretion is reasonably homogeneous in composition, the small increase in core temperature over the past 4.7 aeons implies that radioactive sources are not extremely important; the exact partitioning of core heat between inflow from the accretional process and local radioactives is unknown, but significant contributions from both sources are probable. A relatively small component of heat sources attributable to fossil nuclides has a profound bearing on the problem of the primordial heating of planetary objects as evidenced by early melting in meteorites.

Although ^{244}Pu and ^{129}I are known from xenon traces and fission tracks (for ^{244}Pu) to have existed in the original solar nebula¹⁵, their existence in planetary matter after accretion to regions where heat could be retained is clearly unimportant. We consider it unlikely that this material was strongly fractionated before accretion of the planets with the parent body progenitors of the pallasites and iron meteorites receiving an inordinate share of these isotopes in comparison with the Moon. Assuming no fractionation, there must be another heating mechanism to explain those meteoritic objects which display convincing evidence of fast reheating shortly after the formation of the solar system. Consequently, the hypothesis of ohmic heating, through a strong interplanetary electric field resulting from a premain sequence Sun spinning rapidly and associated with the intense plasma flux characterizing T Tauri stars^{15,16}, is strengthened.

Although a detailed study of the accretional time span and profile is not included here, simple calculations show that, because of the moderate core temperature today, the accretional time span could not have been more than 5,000 yr and possibly much less. An accretional model, together with the magnetometer data, suggests that the lunar core is composed of primordial matter, never melted, and that the interior temperature is rising. We consider the mantle to be a primordial mass which differentiated *in situ* and which should display the differentiate spectrum of an isolated volume which has undergone strong thermal working. The lava flows on the surface then correspond to outflow from this reservoir.

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Estimation of Secondary Structure in Ribonucleic Acids

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A simple method for estimating the secondary structure of an RNA molecule has been proposed on the basis of the knowledge of its sequence.

THE nucleotide sequences of transfer RNA molecules¹, portions of viral messenger RNA molecules²⁻⁸ and ribosomal RNA molecules⁹⁻¹¹ are rapidly becoming known. It is well established that in all RNA molecules, most nucleotide residues interact to form short, intramolecular helical regions and unpaired loops, so defining a secondary structure^{12,13}. In many cases, further folding leads to a unique, biologically active tertiary structure. Even for molecules with known sequences, it is difficult to determine the secondary structure. Optical techniques can only estimate the number and type of residues in helical regions¹⁴⁻¹⁶. Other, more direct, methods often need large amounts of purified material, and frequently necessitate studying the structure in non-physiological conditions¹⁷⁻²⁰.

We propose here a simple method for estimating the secondary structure of an RNA molecule from knowledge of its sequence. This problem was first approached by Fresco,

Alberts and Doty¹². By allowing looped out bases and putting the sequence in register in order to maximize base pairing, they were able to suggest optimal secondary structures from a given sequence. When this approach was applied to transfer RNA sequences, a wide variety of sequences could all be put into a similar cloverleaf pattern with the anticodon in the same position, thus demonstrating the interchangeability of the tRNAs in protein biosynthesis on the ribosome²¹. Knowledge of the function of the molecule helped in the choice of a likely structure. For the 5S ribosomal RNA molecules, the lack of such functional knowledge and the large number of possibilities for intramolecular pairing led to a number of proposed models with no obvious correct choice²²⁻²⁶. Longer sequences are expected to lead to more ambiguous possibilities. More thermodynamic information on the relative stability of the loops and various helical regions is necessary. The estimates of secondary structures presented here are the results of analysis of a limited amount of data on double strand formation in oligonucleotides and complementary polynucleotides.

Rules for RNA Structure

The secondary structure which an RNA molecule takes up at equilibrium in any solution is that which minimizes the free energy of the solution. The free energy, ΔG , of a double

stranded structure relative to the single strand is written as the sum of enthalpy, ΔH , and entropy, ΔS , terms.

$$\Delta G = \Delta H - T\Delta S$$

For simplicity, we shall assume that both ΔH and ΔS are independent of the absolute temperature, T . It is convenient to consider that the formation of a double stranded region occurs in two steps: the formation of the first base pair (initiation) and the formation of subsequent base pairs (propagation). Both steps contribute to the total free energy of helix formation.

The initiation of a base paired region in a single stranded RNA molecule involves the formation of a loop. A stranded representation for the free energy of initiation is²⁷

$$\Delta G (\text{initiation}) = -2.3RT \log \gamma_m$$

where $R = 2$ calories $\text{mol}^{-1} \text{deg}^{-1}$ and γ_m is the probability of the formation of a loop of m unbonded bases. γ_m depends on the identity and number of bases in the loop. As m decreases, the formation of the loop becomes more probable, but finally steric hindrance prevents loop formation.

The expression for the free energy of propagation of a helical region is simplified by the existence of a direct relationship between the enthalpy and entropy of propagation. Because the value of ΔG of initiation for a long double stranded RNA is negligible compared with that of propagation, and the melting point for the polymer, T_m^∞ , is defined as the temperature at which equal amounts of double strand and single strand are in equilibrium, it follows that

$$\Delta G (\text{propagation}) = 0 \text{ at } T_m^\infty$$

$$\Delta S (\text{propagation}) = \frac{\Delta H (\text{propagation})}{T_m^\infty}$$

Therefore, the free energy of propagation for a short RNA double helix at a temperature T is given by

$$\Delta G (\text{propagation}) = \Delta H \left(1 - \frac{T}{T_m^\infty} \right)$$

The value of T_m^∞ used depends on the guanine-cytosine content of the short helix and the ionic strength of the solvent. The value of ΔH , in principle, depends on the chain length, the base composition and the base sequence of the short helix.

The total free energy of double strand formation in an RNA molecule is the sum of initiation and propagation terms for each different helical region.

$$\Delta G = \Delta H \left(1 - \frac{T}{T_m^\infty} \right) - 2.3 RT \log \gamma_m$$

The structure with the lowest total free energy will be the most stable and probably the one actually present.

By studying double strand formation in oligonucleotides and polynucleotides of defined base composition and sequence, values can be obtained for the parameters given here in various solvents, and thus we can extrapolate to any proposed structure. The principal difficulty is that there is only a limited amount of thermodynamic information on suitable models. This means that at present a considerable number of approximations must be made, which can be removed when more data become available.

It is efficient to present the conclusions first and then discuss the experiments which led to them. Information about the stability of double stranded regions came from melting temperature and thermodynamic data on double stranded polynucleotides²⁸ and oligonucleotides^{29,30}. The probability of loop formation has been estimated from the melting curves of DNA³¹, from oligo dAT melting behaviour²⁷ and from work on the formation of loops in $A_6C_mU_6$ (Uhlenbeck

and Borer, unpublished results). The stability of bulges was estimated from the melting temperatures of partially mismatched double stranded polynucleotides^{32,33}. These experiments suggested assignment of the following stability numbers for predicting the most stable secondary structure in an RNA at 25°C in a neutral buffer of moderate or high ionic strength: (1) A-U pairs, +1; (2) G-C pairs, +2; (3) G~U pairs, 0; (4) hairpin loops, -5 to -7; (5) interior loops, -4 to -7; (6) bulges, -2 to -6.

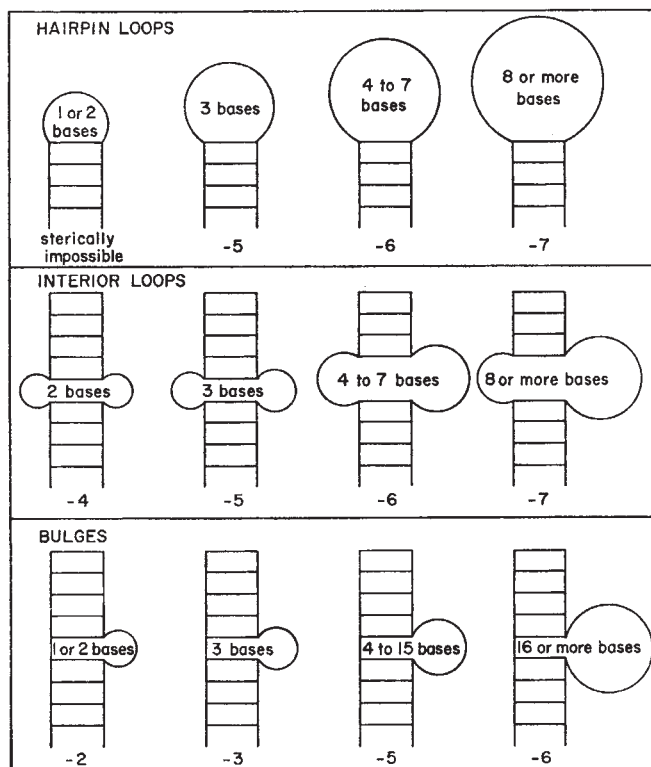


Fig. 1 Giving the contributions of loops and bulges with unbonded bases to the stability of secondary structure in nucleic acids. A positive number represents an increase in stability relative to the single strand; an A-U base pair has a stability number of +1.

The numbers for the different loops and bulges are defined and illustrated in Fig. 1. The stability number for a given RNA secondary structure is the sum of the contributions of the loops, bulges and helices; the structure with the highest number is the most stable. The free energy (and stability number) of the completely single stranded molecule is, by definition, equal to zero. Stable structures have positive numbers measured in units of the free energy of forming an A-U base pair (found to be -1.2 kcalories mol^{-1} at 25°C). A negative stability number characterizes a secondary structure unstable with respect to the single strand. Each base pair formed obviously increases the stability, but each loop decreases the stability. Thus, at least two G-C pairs and one A-U pair are necessary to close a hairpin loop.

As an example, we consider the sequence of fifty-five bases in the coat protein cistron in R17 viral RNA sequenced by Adams *et al.*³. All possible pairs between A-U, G-C and G~U in the sequence can be represented by the base pairing matrix in Fig. 2, in which 1, 2 and 0 represent the respective stability numbers. Adjoining numbers on diagonals leading from the lower left to the upper right represent antiparallel base paired regions. Breaks or jogs in the diagonals represent interior loops or bulges. By joining different base paired regions in the pairing matrix, all possible secondary structures can be investigated and their stability numbers computed.

Although a direct search for likely structures is possible for sequences as short as that considered, the base pairing matrix is useful for longer sequences, and the search for structures and their subsequent evaluation is carried out by computer.

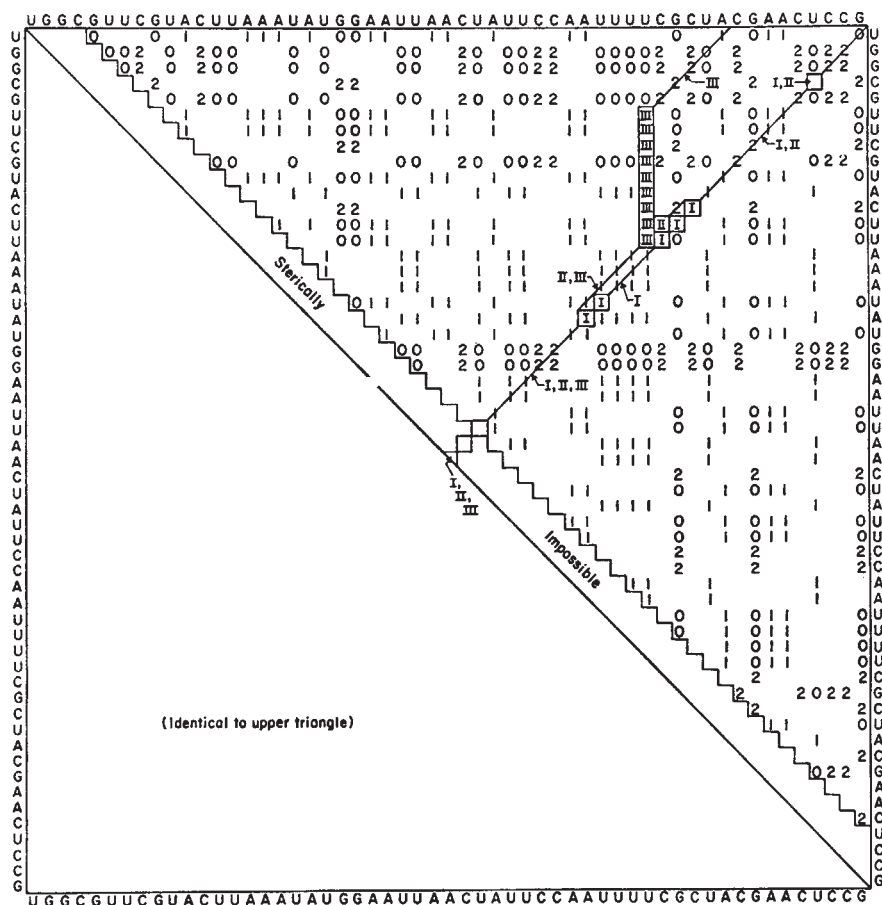
Three possible structures for the 55-mer are shown in Fig. 3 and are represented by diagonal lines joining squares in Fig. 2. Each structure was assigned a stability number (and a free energy), formed by summing the contributions listed. Structure I ($\Delta G = -3.6$ kcalories mol⁻¹) is essentially the one given by the authors who determined the sequence³. A slightly different structure II ($\Delta G = -9.6$ kcalories mol⁻¹), was predicted to be considerably more stable. In fact, in equilibrium at 25° C, the ratio of the concentration of structure II to structure I was calculated to be $10^{6.0/1.36} = 26,000$. Finally, a structure III was calculated to be slightly more stable ($\Delta G = -4.8$ kcalories mol⁻¹) than structure I although it has four fewer base pairs.

Table 1 Stability Numbers of Proposed 5S RNA Models

	5S stability number	No. AU+GC base pairs
Cantor ²⁵	30	43
Lewis ²⁶	25	32
Boedtker and Kelling ²²	17	32
Madison ²⁴	16.5	32
Raake ²³	-22	27

by Lewis and Doty²⁶ seem to be the most stable, other structures can be found which give comparable or even somewhat higher stability numbers. Many of these structures have an additional difficulty; they are highly compact three dimensional arrangements in which the possible destabilizing effect

Fig. 2 The base pairing matrix showing all possible base pairs in a sequence of fifty-five bases from R17 viral RNA³. An upper right to lower left diagonal line joining base pairs represents an anti-parallel helical region. Squares represent loops between helical regions. A bulge is seen as a vertical or horizontal line. The three most stable structures (I, II, III) are indicated here and in Fig. 3.



Simply breaking the three A-U base pairs in the centre of structure I was calculated to improve its stability by 2.4 kcalories. Furthermore, the two G-C base pairs at the bottom of structures I and II are only marginally stable. No gain in stability was predicted by forming them. Similarly, the bottom helix in structure III is marginally stable. This emphasizes the fact that the practice of simply maximizing base pairs to predict secondary structure must be tempered by also minimizing the number of loops.

These simplified stability calculations have been performed for a number of different tRNAs. It is not surprising that the cloverleaf models for tRNAs are essentially the most stable form, although base pairing between the loops of the cloverleaf is sometimes suggested. A comparison of the stability numbers of several proposed structures for *E. coli* 5S RNA is shown in Table 1. Although the structures proposed by Cantor²⁵ and

of double helical regions constrained to be close to each other is not known. Although in this case the method is unlikely to predict an unambiguous stable structure, it is valuable in significantly limiting the number of possibilities. For the longer messenger RNA molecule, in which such complex structures are less likely, the application of the technique is more straightforward. As more data on model compounds are gathered, it should be possible to obtain a more sensitive set of stability numbers.

Thermodynamic Details

The assignment of free energies for the formation of A-U and G-C base pairs is quite direct. The ΔH of formation of double strands by complementary oligonucleotides has been determined for the molecules A_nU_n ($n=4-7$)²⁸, A_nGU_n +

$A_mCU_n(n, m=3-5)$, $A_nCGU_n(n=2-4)^{28}$, and $A_nGCU_n(n=2-4)$ (Uhlenbeck and Dengler, unpublished results) in 1 M NaCl, 0.01 M sodium phosphate buffer, 10^{-4} M sodium EDTA at pH 7. ΔH was evaluated from the concentration dependence of T_m with the equation³⁴

$$\frac{1}{T_m} = \frac{1}{T_m^\infty} + 2.3 \frac{R}{\Delta H} \log \kappa c$$

where T_m^∞ is the melting point of a long polynucleotide with the same average base composition as the oligonucleotide, κ is the equilibrium constant for forming the first base pair between the oligonucleotides and c is the oligonucleotide strand concentration. This equation, which assumes that the formation of the double strand is an all or none process, is entirely adequate for the analysis of the oligomers considered. The data for the fifteen different double strands formed could be fitted by the equation

$$\Delta H = (N-1)(-8 \pm 0.7 \text{ kcalories mol}^{-1})$$

where N is the total number of base pairs. The choice of $(N-1)$ assumes that the formation of the first base pair in the helix has a negligible enthalpy, because it is devoid of a stacking interaction. Although the data suggest that ΔH depends on the base composition of the helix, the variation is not conclusive at the present degree of accuracy. This value of $\Delta \bar{H} = -8$ kcalories for adding a mole of base pairs is consistent with direct calorimetric values (-7.4 to -8.2 kcalories mol^{-1}) for the melting of polyadenylic acid : polyuridylic acid double strands in 0.02 M to 0.1 M Na^+ ³⁵. Also, DNA double strands with G-C contents which varied from 37% to 64% gave a value of -8.0 ± 0.2 kcalories mol^{-1} (ref. 36).

Having assigned a value to the enthalpy, we need only know the values of T_m to estimate the free energy for the formation of A-U and G-C bonds. For natural double stranded RNA, Kallenbach²⁸ has expressed the reciprocal of T_m as a linear function of the G-C fraction, f_G , as

$$\frac{1}{T_m} = \frac{1}{T_A} + f_G \left(\frac{1}{T_G} - \frac{1}{T_A} \right)$$

where T_A and T_G are the melting temperatures of polymers with 100% A-U and 100% G-C base pairs, respectively. The values of T_A and T_G were extrapolated from a series of natural RNA molecules of different G-C content and also agree with the measured T_m s of the appropriate synthetic polynucleotides²⁸. The extrapolated values of T_m obtained from the oligonucleotide data were also consistent with the polymer data. For 1 M NaCl, pH 7, T_A is 78°C and T_G is 152°C ²⁹. Thus, at 25°C in 1 M NaCl, pH 7, the free energies of base pair formation were estimated as:

$$\begin{aligned} \Delta G(\text{addition of A-U base pair}) &= \Delta \bar{H} \left(1 - \frac{T}{T_A} \right) \\ &= -1.2 \text{ kcalories mol}^{-1} \end{aligned}$$

$$\begin{aligned} \Delta G(\text{addition of G-C base pair}) &= \Delta \bar{H} \left(1 - \frac{T}{T_G} \right) \\ &= -2.4 \text{ kcalories mol}^{-1} \end{aligned}$$

With stability units of -1.2 kcalories mol^{-1} we obtained the value of +1 for an A-U base pair and +2 for a G-C base pair (rules 1 and 2). Because the values of T_A and T_G depend on salt concentration, the stability of the G-C pair relative to the A-U pair depends on the solvent. The values given are applicable in high sodium ion concentrations or solvents containing 10 mM magnesium ion or more. At lower ionic strengths, the G-C bond is slightly more stable than the A-U bond. For example, from T_m data in 0.2 M NaCl, we calculated

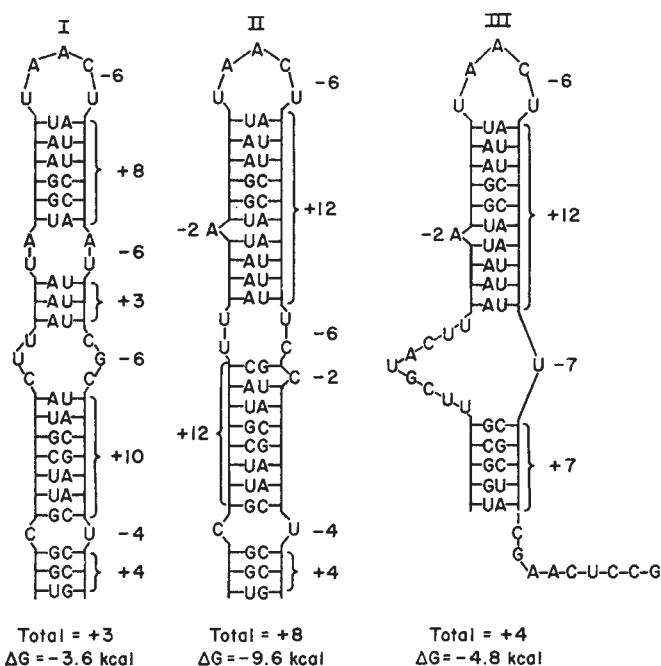


Fig. 3 Gives three possible secondary structures for a sequence of fifty-five bases from R17 viral RNA³. Structure I, a proposed structure³, is calculated to be least stable. Structure II, a slightly different structure, is significantly more stable. Structure III, with fewer base pairs, is slightly more stable than structure I.

$\Delta G(\text{A-U}) = -1.0$ kcalories mol^{-1} and $\Delta G(\text{G-C}) = -2.2$ kcalories mol^{-1} . This difference is, however, not large enough to be significant at the present degree of approximation. The temperature at which the secondary structure is considered also affects the free energies. The ratio of G-C to A-U stability increases with increasing temperature, but is constant, to within 20%, in the temperature range 0° to 40°C .

The number zero for G~U (rule 3) came from experiments on double strand formation in $A_nGUm(n, m=4,5)$ and $A_nUGUn(n=4,5)$ ³⁰. The data are consistent with having G~U bonds in the helix with either zero or slightly negative stability, rather than G forming a bulge in the A-U helix.

Experimental data for the free energies of loops are scarce, and essentially no work has been done for RNA as a function of the number and composition of bases in a loop. Some insight into the stability of loops, however, can be gained from chain statistics. Bases in a loop are less stable than the same bases in a single strand because the ends of the loop are fixed relative to each other, thereby decreasing the entropy of the chain and increasing the free energy. For a chain of m bases, the end to end distances in the single strand of which have a Gaussian distribution, the free energy of a loop relative to the single strand has the form³⁷

$$\Delta G(\text{initiation}) = -2.3RT[B - 1.5 \log(m+1)]$$

where B is a parameter which characterizes the formation of the first base pair which closes the loop. The free energy depends on the number of statistical links to the -1.5 power³⁸. We equated the number of statistical links to the number of unbonded bases plus one, $(m+1)$, but for a more realistic chain, in which the end to end distances are corrected for excluded volume (that is only self-avoiding conformations are counted), the factor of 1.5 was replaced by 1.75³⁹. The important conclusion from this equation is that, to the extent that entropy determines the loop stability, the free energy changes slowly with loop size; a loop of ten bases is only about 0.5 kcalories mol^{-1} less stable than one of six bases. This conclusion applies equally to interior loops. For small loops (m less than four or five bases), the free energy equation given

here is no longer applicable because significant deviation from Gaussian behaviour is expected and stacking interactions between the residues in the loop may play a considerable role.

It remains to estimate B in order to assign a stability to loops. As a first approximation to RNA loops, we consider the values of B deduced from experimental results for DNA. Crothers and Zimm³¹ reported $B = -3.5$ and -3.9 from an analysis of the melting curves of dAT:dAT and dI:dC Crothers⁴⁰ obtained a value for B of between -3 and -4 for T2 phage DNA. Scheffler *et al.*²⁷ found $B = -2.35$ for oligo dAT loops with $m \geq 6$. For complementary double stranded RNA oligonucleotides, we have calculated values of B between -2 and -4 by assuming a correlation between intra and intermolecular initiation processes. A value of B of approximately -3.4 fits our preliminary data for the T_m of $A_6C_mU_6$. We therefore assigned an average value of -3 to B for loops with four or more bases.

We consider now loops with fewer than four bases. Space filling models show that it is impossible to make a hairpin loop with one or two bases. For the minimum hairpin loops of three bases, we estimated the free energy by analogy with the finding of Scheffler *et al.*²⁷, that the minimum loop is about 1.2 kcalories mol^{-1} more stable than expected from the equation for large loops. This same correction was applied to interior loops of three bases.

Unlike the case of hairpin loops, interior loops of two residues are sterically possible and correspond to two mismatched bases (not A-U, G-C, or G~U) on opposite strands. The oligonucleotides A_nCU_n ($n=4, 5, 7$) which form dimers in solution are examples of such loops¹⁸. If we attribute the differences in T_m for these molecules from the corresponding A_nU_n series solely to ΔG (initiation) of the interior loop, we estimate a value of about $+5$ kcalories mol^{-1} for the formation of interior loops of two bases. This result is consistent with the expected stability of small loops over that of the prediction for Gaussian chains.

Bulges of any number of bases are sterically possible. By analysing the mixing curve for the complex formed between poly U and poly (A, U), Fresco and Alberts⁴¹ showed that bulges are formed in preference to interior loops. This result probably reflects the fact that the formation of an interior loop involves an interruption of the helical region, thus causing the loss of a stacking interaction. This does not occur in the formation of a bulge, because one strand is not interrupted. Other workers have shown that U is not unique in its ability to form small bulges^{32,33,42,43}. A rough estimate of the ΔG (initiation) can be obtained from the reported melting temperature of the poly U+poly (A, U)³² and poly U+poly (A, I)³³ complexes, if the simplifying assumptions are made that the copolymer sequences are random and that each bulge may be treated as though it were of average size, determined from the fraction of mismatched base in the copolymer. At a mole fraction of 0.1 I in poly (A, I) the average bulge in the complex has slightly more than one base; this yields a value of ΔG (initiation) of $+1.9$ kcalories mol^{-1} at 25°C . At a mole fraction of 0.47 U in poly (A, U), the average bulge in the complex has nearly two bases, but the ΔG (initiation) value is still found to be about $+1.9$ kcalories mol^{-1} .

With the various contributions of helix content, loops and bulges thus estimated, we can now write the expression for the free energy of forming a secondary structure relative to the single strand, as

$$\Delta G = N_{AU}\Delta\bar{H}\left(1 - \frac{T}{T_A}\right) + N_{GC}\Delta\bar{H}\left(1 - \frac{T}{T_G}\right) - \Delta\bar{H}\left(1 - \frac{T}{T_m^\infty}\right) - 2.3 RT[B - 1.5 \log(m+1)]$$

The enthalpy for the formation of one base pair, $\Delta\bar{H}$, is taken to be -8 kcalories mol^{-1} . The first two terms are the free energies of adding N_{AU} A-U and N_{GC} G-C base pairs. The

third term takes into account the fact that the free energy of a double strand region depends on $N-1$ rather than N . Each double strand region has a different T_m^∞ associated with it because of variation in the fraction of A-U and G-C base pairs, but we used an average value of 1.8 kcalories mol^{-1} for this term. The fourth term depends on B , which we chose equal to -3 , and on the number of unbonded bases, m , in each loop or bulge. As there must be one loop (not bulge) for each double strand region, we can combine the third term with the factor of $-2.3 RTB$ from the fourth term. Thus, at 25°C we obtain ΔG in kcalories mol^{-1} as

$$\Delta G = -1.2 N_{AU} - 2.4 N_{GC} + 5.9 + 2.0 \log(m+1) \text{ (for each loop of at least four bases)}$$

or

$$+ 4.1 + 2.0 \log(m+1) \text{ (for each bulge of at least four bases)}$$

Dividing all the free energies in this expression by -1.2 kcalories mol^{-1} to transform them into A-U base pairing units, we obtain the numbers (rounded to the nearest integer) given in Fig. 1 for loops and bulges with four or more residues (rates 4-6 in the text). For loops of three bases and bulges of one, two, or three bases, the different values discussed earlier are given in Fig. 1.

We present these estimates of the energetics of forming secondary structure in RNA molecules on the basis of the present, limited data for the formation of loops, bulges, and short helices. More detailed analysis would consider the effect of temperature, ionic strength and base sequence on both ΔH and ΔS . In particular, the sequence of bases in a loop or bulge is likely to be important, because single stranded stacking should have an effect on the stability of such structures⁴⁴. The partitioning of the free energy of the helical region into that of the formation of A-U, G-C and G~U pairs is a simplification, because the sequence dependent stacking between base pairs is the principal source of stability within the helix. Possible non-Watson-Crick base pairs were excluded from consideration and interaction among loops and helical regions within the structure was ignored. In spite of the approximations involved in the assessment of the thermodynamic parameters and the scarcities of data on loops and bulges, these rules should provide a useful framework for the evaluation of RNA secondary structures.

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Induction of Directional Chromosome Elimination in Somatic Cell Hybrids

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The chromosomes of one or other of the "parents" of a hybrid cell are usually gradually lost over successive generations. X-irradiation, γ -irradiation or BUDR labelling of one "parent" before fusion can be used to predetermine which of the two sets of chromosomes will be preferentially lost.

THE fusion of somatic cells of different species produces hybrids which, in most cases, preferentially lose some or all of the chromosomes of one of the parental species on prolonged subculture. So far, however, there has been no general way of predetermining which of the parental sets will be preferentially reduced. I report here a technique to this end in the belief that it will constitute a useful tool in the use of somatic cells for formal genetic analysis.

The idea stems from work carried out more than 30 years ago¹⁻³. The problem was how to obtain individuals recombinant with respect to whole chromosomes from two species of *Drosophila* the hybrids of which were sterile so that the recombinants desired could not be produced by means of meiosis and fertilization. The solution was to X-irradiate the haploid sperms of one species and then to use these to fertilize eggs from triploid females of the second species. Triploid females produce a high proportion of eggs with one or more chromosomes in duplicate ("disomic"). Irradiation induces potential breaks in the sperm chromosomes and their subsequent elimination in the cleavage divisions of the zygote. An egg, disomic for a chromosome homologous to one broken in the spermatozoon which fertilized that egg, would give rise to

a diploid zygote carrying exclusively the two maternal chromosomes in question. The same procedure was applied successfully at the intraspecific level by using triploid females with multiple recessive genetic markers and diploid males of the same species with the corresponding dominant alleles⁴.

The problem of inducing a preferential elimination of chromosomes from interspecific or intraspecific mammalian somatic cell hybrids is similar. In both cases there is redundancy in the chromosome sets and loss of chromosomes is not necessarily lethal. A partial solution for interspecific cell hybrids came in 1967 when Weiss and Green⁵ discovered that hybrids between somatic cells of mouse and man would gradually lose the human chromosomes on continued culture. Cell lines would therefore eventually arise which had retained only one or a few human chromosomes. By this means human genes can be assigned to individual human chromosomes and linkages recognized or excluded⁶⁻⁹. A similar elimination occurs in Chinese hamster-man hybrids¹⁰. This directional elimination of chromosomes from interspecific hybrids is very useful for locating genes specifying those human proteins which can be distinguished from their mouse, or Chinese hamster, counterparts. It is of little use, however, for locating human genes known only because of the effects of recessive alleles.

Irradiation of Parent Cells

The technique described here allows the experimenter to choose which of the parental chromosome contributions shall be preferentially eliminated in hybrid somatic cells. It involves X or γ -irradiation of the cells of one "parent" before they are fused with those of the other. An alternative to X-irradiation is to label the chromosomes of one "parent" with bromodeoxyuridine (BUDR) and thus sensitize them to visible light^{11,12}.

With the first assay system I used, the loss from hybrid clones of the chromosomes of the treated "parent" can be detected unmistakably. All but one of the chromosomes of

one parental cell—Chinese hamster *wg 3 IMP⁻*—are morphologically quite distinct from those of the other—mouse *3T3 TK⁻*. Furthermore, this combination of strains yields hybrid cells which, on prolonged culture, lose “spontaneously” only very few chromosomes and this loss is not markedly preferential for either species (but see ref. 13). Thus the loss of chromosomes of either species can be detected by morphological classification and count of the chromosomes in the cells of individual hybrid clones each originated from an independent union (Figs. 1 and 2).

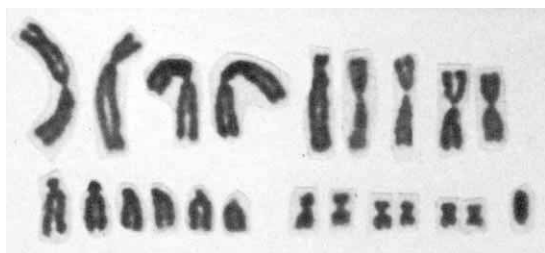


Fig. 1 A typical karyogram of Chinese hamster strain *wg 3 IMP⁻*: twenty-two chromosomes, all but one (bottom right) distinguishable from those of mouse strain *3T3 TK⁻* (bottom three rows of Fig. 2).

wg 3 IMP⁻ cells are Chinese hamster fibroblasts deficient in inosine monophosphorylase activity and resistant to 2×10^{-5} M 8-thioguanine, with a pseudodiploid chromosome number of twenty-two. *3T3 TK⁻* cells are mouse fibroblasts deficient in thymidine kinase activity and resistance to 10^{-4} M BUdR, with a quasi triploid complement of sixty-four to sixty-eight chromosomes (mode: sixty-six). A small proportion of cells of both strains have double these chromosome numbers. Littlefield's¹⁴ selection technique using HAT medium can be applied to the isolation of hybrids between the two strains: neither can grow in HAT, but their hybrids can. The doses of irradiation used (80 to 1,600 r.), the way—in suspension or on monolayers¹⁵—in which cells are fused with the use of Sendai virus¹⁶, inactivated by β -propiolactone¹⁷, the amount of virus used, the density of plating, the number of days after fusion for the isolation of hybrid clones (10–30), and the species used, were all varied while the technique was being developed. A standardized procedure is given here.

A 2 day old monolayer culture of 10^5 cells of one strain growing in a 5 cm Petri dish in Dulbecco's medium with 10% foetal calf serum is washed twice with medium without serum and chilled to about 4° C. The medium is then replaced with 0.5 ml. of chilled inactivated Sendai virus suspension (1,000 HAU) in saline buffer. After 10 min in the refrigerator the unadsorbed virus is washed off with cold medium lacking serum and a chilled suspension of 10^5 cells of the second strain in 0.5 ml. is added. The Petri dishes are kept in the refrigerator for 15 min and occasionally rocked to facilitate the agglutination of the cells in suspension to those of the monolayer which had preadsorbed the virus. The dishes are then transferred to 37° C for 10 min to let fusions occur, the cells not stuck to the monolayer are removed by washing with HAT medium (10% foetal calf serum) and the dishes are replenished with this medium which is changed subsequently every 3 days. By the fifth day small colonies of presumed hybrids begin to appear, and are marked, while the parent cells gradually die. By the tenth day the hybrid clones are large enough to be isolated. They are subcultured in HAT. By the twentieth day most have multiplied sufficiently to permit the preparation of karyograms.

Irradiation (X-rays: 100 kV, 4 mA, 2 mm Al, dose rate 200 r./min or γ -rays from a cobalt bomb) is preferably carried out on monolayers; 600 r. is a suitable dose. This dose is known to induce a mean of 2.5 observable chromatid breaks or exchanges per cell in Chinese hamster fibroblasts¹⁸ and to leave

about 15% of the cells capable of forming colonies. Table 1 summarizes the results of an experiment with this dose of irradiation. Chromosome counts and classification are based on the analysis of four to six karyograms for each clone 60–70 days after fusion. In this experiment the irradiated cells of either parent were exposed to trypsin and added as a suspension on top of the monolayers of cells of the other parent which had not been irradiated but had been exposed to Sendai virus. Reversing this combination of treatments does not seem to matter.

Table 1 Chromosome Counts on Hamster–Mouse Hybrid Cells with One Irradiated Parent

Hybrid clone	Irradiated hamster (22 chromosomes)		Irradiated mouse (mode 66 chromosomes)		Hybrid clone
	Chromosomes lost Hamster	Mouse	Chromosomes lost Hamster	Mouse	
3T3/wg-b *	7.8	–1.8	1	4.5	3T3/wg-a *
3T3/wg-c	7.8	1.6	2.4	9.2	3T3/wg-b
3T3/wg-d	3.6	–2.0	1.2	3.3	3T3/wg-f
3T3/wg-e	3.0	0	3.4	3.2	3T3/wg-g
3T3/wg-g	3.4	0.4	1.5	–2.7 †	3T3/wg-h
3T3/wg-h	4.8	0.5	1.5	5.0	3T3/wg-i
3T3/wg-i	3.8	–0.2			
3T3/wg-k	3.2	0.7			
Mean	4.7	–0.1	1.7	3.7	Mean

* Irradiated “parent”.

† One clone, out of fourteen, in which the loss was in the opposite direction to that expected.

Survival of Unirradiated Chromosomes

Irradiation with 600 r. of the hamster cells induced subsequent elimination from individual hybrid clones of up to six hamster chromosomes in addition to those lost even without irradiation (about two). Irradiation of the mouse cells induced elimination of up to nine mouse chromosomes. Hybrid clones from non-irradiated cells of these two strains retain about twenty hamster and sixty-five mouse chromosomes after 2 months in HAT medium. In the selective system used, the retention by a hybrid clone of at least one mouse chromosome and one hamster chromosome is a condition for survival. The

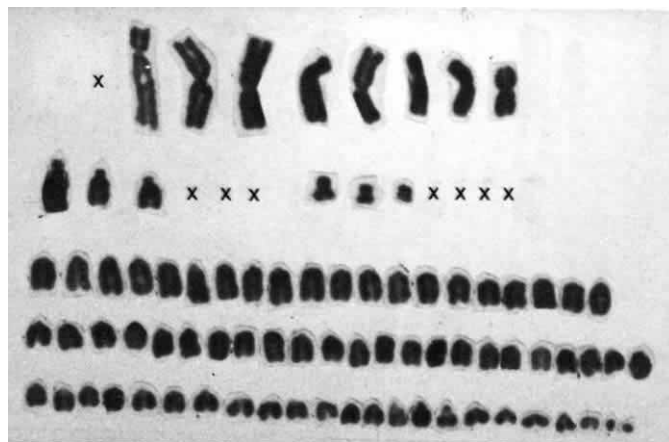


Fig. 2 Karyogram of a hybrid clone from irradiated (600 r.) hamster cells. The hamster chromosomes are arranged in the top two rows in the same order as in Fig. 1; eight, indicated by X, are missing. The sixty-six mouse chromosomes in the lower three rows correspond to the modal complement of mouse strain *3T3 TK⁻*.

hybrid clones which become established in this system represent therefore a selected sample of karyotypes with fewer chromosome losses than if selection were not applied.

Larger doses of irradiation produce more extensive chromosome losses, but entail several disadvantages. A proportion of hybrid clones gradually dies and chromosome aberrations are more frequent. Furthermore, among surviving clones there are many with multiple sets of chromosomes, chiefly from the non-irradiated parent. It is not clear what proportion of these surviving clones with multiple sets of chromosomes of one parent originated from multiple fusions (for example, two mouse cells and one hamster), initial or in succession, or from fusions involving a polyploid parental cell. As polyploid cells constitute at most 10% in the two strains used, the proportion of hybrids with a polyploid contribution from one parent, obtained when high doses of irradiation are used, is much higher than expected from the proportion of pre-existing polyploids.

For example, in an experiment in which either parent was irradiated with 1,000 r., of thirteen established clones from irradiated mouse cells, five had a tetraploid hamster complement and a variably reduced mouse complement (minimum in one clone: forty-two). Conversely, of eight established clones from irradiated hamster cells, five had a mouse complement of 120 or more and a reduced hamster complement (minimum in one clone: eleven), two had about normal mouse complements and reduced hamster complements, and one had a normal mouse complement and a less than tetraploid hamster complement. This last hybrid probably originated from fusion of a hamster cell with forty-four chromosomes, many of which were later lost, with a normal (about sixty-six chromosomes) mouse cell.

In an experiment with the highest dose of γ -rays used so far—1,600 r.—no hybrids became established from irradiated hamster cells, and only three clones from irradiated mouse cells. They all carried an almost complete tetraploid hamster complement and only twenty to thirty mouse chromosomes.

All this suggests that when—as after heavy irradiation—one chromosome complement becomes very imbalanced, hybrids with a multiple contribution from the unirradiated parent survive preferentially. The imbalance in this case is relatively lessened.

BUDR Labelling

As an alternative to X or γ -irradiation, the cells of one parent can be grown in the presence of BUDR for one or two replication cycles and then exposed to visible light, which induces breaks in chromosomes which have incorporated BUDR^{11,12}. The combined BUDR and light treatment is therefore equivalent to irradiation with ionizing radiations. It offers, however, the valuable possibility of delaying exposure to light until after fusion, thus avoiding possible effects on the procession of fusion itself. (I am indebted to Dr G. Marin for this suggestion.) Only the chromosomes of the BUDR-labelled parent will then be sensitive to light within the hybrid nucleus or the heterokaryon. Furthermore, the BUDR technique offers the possibility of selective incorporation into, and therefore sensitization of, individual chromosomes according to their time of replication. For this purpose, and not for others, it may well be a more useful technique than that based on ionizing radiations.

The experiments with BUDR and light are so far only preliminary but they suggest that this treatment is as effective as ionizing radiations. After growing *wg 3 IMP*⁻ for 20–22 h in Dulbecco's medium with 10% dialysed foetal calf serum and 10^{-5} M BUDR, hybridizing the cells with 3T3 TK⁻ and illuminating with visible light the monolayers (in phosphate buffer saline) 20–24 h after hybridization, considerable elimination of hamster chromosomes occurs. The treatment used is equivalent to that giving a 5% survival point on the curves published by Puck and Kao¹². Fig. 3 shows a metaphase in a mass culture of hybrids, 30 days after fusion, treated as above.

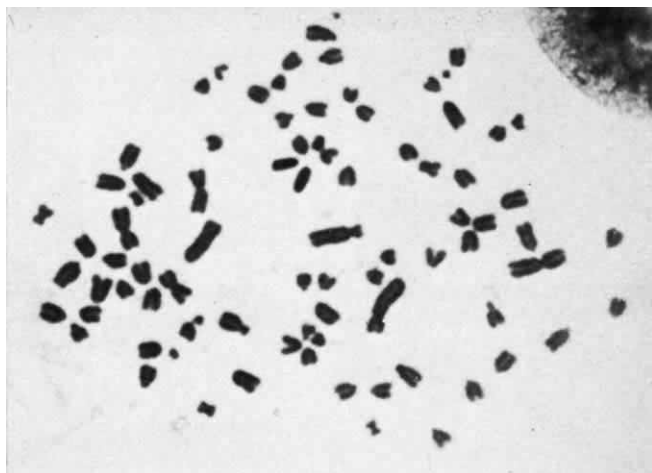


Fig. 3 Metaphase plate from a mass culture of hybrids grown for 30 days in selective medium (HAT). The Chinese hamster *wg 3 IMP*⁻ parental cells were labelled with 10^{-5} M BUDR for 22 h before hybridizing them to the mouse cells 3T3 TK⁻. Light treatment was given 24 h after hybridization. This hybrid cell shows a modal complement of mouse chromosomes (sixty-six) and a reduced (seventeen) complement of hamster chromosomes.

Of the hamster chromosomes, which were BUDR labelled, seventeen remained together with a full complement of mouse chromosomes.

Experiments with human–mouse hybrids are in progress to establish whether: (a) treatment of the mouse chromosomes, either by ionizing radiations or by BUDR and light, will reverse the usual elimination of human chromosomes from mouse–man hybrids, and (b) pulse labelling with BUDR of synchronized human cell cultures may achieve differential labelling and therefore preferential elimination of specific human chromosomes.

Clearly, both the X or γ -rays and the BUDR techniques can be applied to intraspecific (for example, man–man or mouse–mouse, and so on) cell hybrids between pairs of genetically marked primary or secondary explants.

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Fanconi's Anaemia in the Genetics of Neoplasia

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By studying relatives of individuals homozygous for Fanconi's anaemia, a method has been developed for determining the effect of heterozygosity for this gene on susceptibility to cancers.

In experimental animals, genetic susceptibility has been shown to be important in determining the frequency and type of neoplasm for both spontaneous tumours and those induced by chemical carcinogens or oncogenic viruses¹. The analysis of genetic factors in human oncogenesis has been much more difficult. There is convincing evidence that close relatives of patients with breast, colonic, or gastric carcinomas are more likely than controls to develop a cancer in the same organ². There are also "cancer families" with an unusual prevalence of malignant neoplasms, often multiple or earlier in onset than similar tumours in the general population^{3,4}. Familial clusters of neoplasms, of one or several different types, can be explained by a strong genetic predisposition, unusual environmental factors, or both influences in varying proportion. In these "cancer families" there is at present no way to detect or characterize a neoplasia-predisposing gene or set of genes in an individual before he is afflicted.

For the specific autosomal dominant or autosomal recessive syndromes associated with malignant neoplasms, gene carriers can be identified by the stigmata of that syndrome. Typical autosomal dominant syndromes in this category are naevoid basal cell carcinoma syndrome, familial polyposis coli and the most common, neurofibromatosis, which has a prevalence of 1 in 3,000 (ref. 5). The identified autosomal dominant syndromes are therefore not numerically important in genetic predisposition to cancer and leukaemia in man. The autosomal recessive syndromes, in which malignant neoplasms frequently occur, are even rarer and include Fanconi's anaemia, ataxia telangiectasia, Bloom's syndrome, xeroderma pigmentosum, Werner's syndrome, dyskeratosis congenita and the Chediak-Higashi syndrome.

Although the affected homozygotes for these autosomal recessive neoplasia-predisposing syndromes are rare, the clinically normal heterozygous carriers occur relatively frequently. For each syndrome, the estimated frequency of heterozygous carriers is between 1 in 200 and 1 in 1,000 in the population. It is therefore important to know whether any of the genes for these syndromes can significantly predispose its heterozygote to develop cancer or leukaemia.

In principle, such an effect of a specific gene might be measured by studying the incidence of death from malignant neoplasms in a set of obligatory heterozygotes—the parents and offspring of affected homozygotes. But because most homozygous probands are young, they have no offspring and their parents are usually alive. To collect enough information it is necessary to include those close relatives whose prior probability of carrying the gene (as a heterozygote) is substantial, although less than one. If the gene is associated with a large increase in cancer and leukaemia mortality in heterozygous carriers, this effect will be detected by analysing the causes of mortality for a set of close relatives of homozygous probands. The prior probabilities of heterozygosity can then be used to estimate how large an increase in mortality from malignant neoplasms is associated with the gene.

The prior probabilities for the relatives of an affected homozygote are computed on the assumption that the gene was present in two of the eight great-grandparents. When the parents of a proband are first cousins, it is almost certain that the gene was present in the grandparents who were sibs and therefore in one of the two great-grandparents in this line. Relatives outside the consanguineous line are considered to have a prior probability of carrying the gene of almost zero. If the parents are unrelated, the prior probabilities of heterozygosity are: parents, 1.0; clinically normal sibs, 0.67; grandparents, aunts and uncles, 0.5; great-grandparents, grandparents' sibs and first cousins, 0.25; and nephews and nieces, 0.33. If the parents are first cousins, the probabilities in the consanguineous line are: parents and grandparents, 1.0; clinically normal sibs, 0.67; great-grandparents, grandparents' sibs, aunts and uncles, 0.5; nephews and nieces, 0.33; and first cousins, 0.25. First cousins once removed have a prior probability of 0.125.

Fanconi's Anaemia

Fanconi's anaemia (FA) was the first syndrome studied, because there is some clinical and laboratory evidence which suggests that it is unusual for the FA heterozygote to develop a malignant neoplasm. The FA homozygote is identified by the combination of short stature, dark skin pigmentation or café-au-lait spots, diverse congenital anomalies and a progressive hypoplastic pancytopenia⁶. Because lymphocyte and fibroblast cultures from most, if not all, FA homozygotes have frequent chromosome breaks and rearrangements^{7,8}, this cytogenetic discovery confirms the diagnosis when clinical features suggest it. Although many of the reported cases died in childhood or adolescence from the pancytopenia, there have been two reports of acute leukaemia⁹, two of monocytic

Table 1 Number of Deaths (and Deaths from Malignant Neoplasms) in Relatives of Patients with Fanconi's Anaemia

Age	1930-5	1935-9	1940-4	1945-9	1950-4	1955-9	1960-4	1965-	All dates	From a malignant neoplasm
0-4	2	1	-	1	-	-	-	-	4	-
5-9	-	-	-	1 (1)	-	1	-	-	2	1
10-14	-	-	-	-	-	-	-	-	0	-
15-19	-	-	-	1	-	-	1	-	2	-
20-24	1	1	-	1	-	-	-	-	3	-
25-29	-	-	-	-	-	-	-	-	0	-
30-34	-	-	-	-	-	-	-	1	1	-
35-39	-	-	-	-	1	1	1	-	3	-
40-44	1	2	-	-	2 (1)	1	-	1	7	1
45-49	-	-	-	1	-	-	-	-	1	-
50-54	2	-	-	1 (1)	1 (1)	-	2 (1)	1 (1)	7	4
55-59	1	1	-	5 (1)	3 (1)	1 (1)	2 (1)	1 (1)	14	5
60-64	1	1 (1)	-	4 (3)	1	5 (2)	1	1	14	6
65-69	1	1	-	-	4 (1)	2 (2)	2	4 (1)	14	4
70-74	-	2 (2)	-	1	1 (1)	-	1	5 (1)	10	4
75-79	-	-	2 (1)	2	1	1	3	3 (1)	12	2
80-84	1	-	-	-	2	1	-	1	5	-
85+	-	1	1	-	-	-	1	-	3	-
All ages	10	10	3	18	16	13	14	18	102 Total	
From a malignant neoplasm	0	3	1	6	5	5	2	5		27 Total

Numbers of deaths from malignant neoplasms are in parentheses next to the deaths from all causes in each square.

leukaemia⁷ and five of young adult FA patients with diverse solid tumours¹⁰.

The FA heterozygote has none of the clinical or cytogenetic abnormalities of the homozygote. In anecdotal reports of cases of Fanconi's anaemia there are several examples of near relatives dying of leukaemia^{7,9} who did not have Fanconi's anaemia and were, if they carried the gene, FA heterozygotes. More recently, in cell culture assays of transformation by the oncogenic virus SV40, cells from both FA heterozygotes and FA homozygotes were transformed much more readily than normal cells¹¹.

Methods Used

Patients with Fanconi's anaemia were sought by writing to all members of the New York Society for the Study of Blood. Each physician reporting a case was asked, after ascertainment, to provide detailed clinical information about the proband and to enlist the cooperation of the family.

A family was studied only if the diagnosis of Fanconi's anaemia was unequivocal in the proband in that he had one or more of the typical congenital malformations in addition to pancytopenia and short stature. The diagnosis also required confirmation in all living probands by the presence of the characteristic chromosome breaks and rearrangements in lymphocyte cultures.

The pedigrees were constructed after interviewing the parents of probands and making inquiries among other family members. All relatives with a prior probability greater than 0.1 of being heterozygous for the FA gene were included, except those branches of the family living abroad or out of contact with the proband's parents for over 20 years. Death certificates were sought for all relatives within this time who died in the United States or Canada, by writing to the appropriate state, municipal, or provincial office. After obtaining the necessary releases, hospital records of the deceased relatives and of the major illnesses of living relatives were also collected. FA homozygotes were excluded from all data and analysis.

The underlying cause of death was abstracted from each

death certificate and noted as due to malignant neoplasm or not, strictly according to the rules of the 1957 World Health Organization *Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death*¹². If the hospital records reported a type of cancer different from that on the death certificate, the tissue diagnosis was used to record the type.

Because of technical limitations in studying control families, fewer cancers have been found in such controls than would be expected from routine mortality statistics^{13,14}, and therefore no control families were studied.

The number of deaths from malignant neoplasms expected in our sample if it were normal was computed by the method of Penrose, Mackenzie and Karn¹³. For this computation all the verified deaths were arranged by year of death and age at death, both in 5 year intervals. In each interval and for each age group, the proportion of deaths from malignant neoplasms was computed from the United States mortality statistics¹⁵ for that period and age group, and the expected number of deaths from malignant disease obtained by multiplying the total number of deaths in that group and time period by this fraction. The sum of all these products is the expected number of deaths from malignant neoplasm in our sample, if normal. Because all the families studied were white, the mortality statistics for the white population were used.

Case Material

The diagnosis of Fanconi's anaemia was established in patients from thirteen families living near New York City. Five of these families were excluded from genetic study for one of these reasons: (1) most of the family lived abroad or were killed in the Second World War; (2) the proband had died very recently; (3) at the time of the study, the mother of an FA child was pregnant and very worried about the outcome; (4) the proband was born of an incestuous mating. The probands from five of the eight remaining families were alive at the time of this study; lymphocyte cultures from these living probands and cultures previously prepared from another

proband, now dead, showed the typical chromosome breaks and rearrangements. In one family of the eight, the parents were first cousins.

Deaths from Malignant Neoplasms

In the eight families studied, there were 106 deaths since 1930 in the United States and Canada of relatives whose prior probability of FA heterozygosity was greater than 0.1. Two of those relatives belonged to long-separated branches of their family and two had died in the Second World War. Of the other 102, death certificates were found for 99. From anecdotal evidence it appeared likely that the other 3 deaths were not due to malignant neoplasms, and they were so recorded. The age of death and the year of death, in five year intervals, are shown in Table 1 for the 102 deaths in this sample.

A malignant neoplasm was the underlying cause of death in 27 cases; the distribution of these deaths by year of occurrence and by age at death is also shown in Table 1. For twenty of the twenty-seven deaths from cancer or leukaemia, a hospital record was available to supplement the diagnosis on the death certificate, and confirmed cancer as the cause of death in every case. In one case, however, the death certificate named rectal carcinoma when the patient had, in fact, a pancreatic carcinoma. For four patients who died of cancer or leukaemia in a hospital, or following hospitalization, the hospital records were unobtainable, and for three others the death certificates were based neither on a hospitalization nor an operative procedure. There was only one case of multiple primary tumours: breast cancer treated with simple mastectomy and irradiation at the age of 45, with death at 56 from endometrial carcinoma. Among the deaths not attributed to neoplasia was one case of a metastatic tumour in the neck several years before death, one of a carcinoma of the breast operated on one year before death, and one case in which a small carcinoma of the rectum was discovered just before death from cardiac causes.

In a normal population, 17.4 ± 3.8 ($\pm$ s.d.) deaths from malignant neoplasm would be expected in 102 deaths, distributed by age at death and year of death as in Table 1. The difference between the observed number, 27, and the expected number, 17.4, is significant at the 0.05 level, employing a 1×2 χ^2 test with Yates's correction.

The deaths from malignant neoplasms in our families occurred predominantly from the age of 50 to 74, as in the population at large. Because the number of cancer deaths in the FA families was greater than normal, there was a relative excess of deaths at ages 50 to 69 in the overall distribution of ages at death from all causes, compared with the normal population. The median age at death for men in the FA families was 63.5, for women, 61. In 1953 (the median year for deaths in our sample), the corresponding population medians were 66.3 and 71.0.

Types of Malignant Neoplasms

Table 2 gives a list of the malignant neoplasms which occurred in the eight families, excluding the probands. As well as those associated with deaths as already mentioned, there are included eight cases of living relatives who had had operations for carcinomas or sarcomas, among the 372 living relatives for whom information about operations and major illnesses was obtained. Several types of neoplasms occurred with unusually high frequency, although the numbers in each category are too small to demonstrate a statistically significant difference from the normal expected value. Three cases of leukaemia were found, where one would be expected. Two were in one family, acute myeloblastic (or monomyeloblastic) leukaemia in an uncle who died aged 58 and acute lymphatic leukaemia in a first cousin who died aged 5, of an FA proband. In a normal population, perhaps two each of colon and stomach carcinomas would be found in 102 deaths. There

were five and four respectively in our sample. The paternal grandmother and the maternal grandfather of one FA proband died from proven gastric carcinomas, while the three pharyngeal carcinomas were in members of three different FA families.

Table 2 Types of Malignant Neoplasms in Near Relatives of Patients with Fanconi's Anaemia

Malignant neoplasm as cause of death	In relatives dying of other causes
Carcinomas	2 breast carcinomas
5 colon	1 rectal carcinoma
4 stomach	1 undifferentiated tumour in the neck
1 rectum	
2 pancreas	In living relatives
3 tonsil, base of tongue	Carcinomas
3 bronchogenic	2 breast
2 breast	2 bladder
1 endometrium	1 endometrium
1 bladder	1 skin
Other neoplasms	
2 acute lymphatic leukaemia	Other
1 acute myelogenous leukaemia	1 leiomyosarcoma
1 Hodgkin's disease	1 myosarcoma
1 brain tumour	

Because it is not possible to distinguish FA heterozygotes from non-carriers in the FA families, there is no way to tell from the present data whether specific types of cancer or leukaemia are associated with the FA gene. The lethal malignancies in the FA families were mostly of the same type as those which are the most frequent cancers causing deaths in the general population. These eight FA families did not have the frequent multiple primaries or the very early age of onset of "cancer families"³ and the tumours were usually different in type from those in "sarcoma families"⁴.

Previous studies of the familial incidence of malignant disease have shown a type-specific or organ-specific increase in malignancies in close relatives of patients with chronic lymphatic leukaemia¹⁶ and cancer of the breast, colon¹⁷, or stomach¹⁸. No overall increase in deaths from malignant neoplasms of all types was found in these studies or among families of children with acute leukaemia¹⁴, in contrast to the present report.

Five of the living relatives had acute poliomyelitis and seven others survived diverse non-bacterial meningitides and encephalitides.

Risk of Death

We have estimated the risk that an FA heterozygote dies from a malignant neoplasm. Each of the 102 relatives who died had a substantial prior probability of carrying the FA gene, because of his relationship with the proband. The number of heterozygotes (N_h) in the sample is estimated by

$$N_h = \sum_{p=0.1}^{1.0} p \cdot N_p$$

where p is the prior probability for the gene, and N_p is the number in the sample with prior probability p . The numbers of deaths at each probability level are given in Table 3. The estimated number of FA heterozygotes among the 102 relatives who died is thus 31.5 ± 4.35 ($\pm$ s.d.), and 70.5 (that is, $102 - 31.5$) were likely not to carry the FA gene. Because 17.4 deaths from neoplastic disease are expected among 102 deaths in a normal population, $70.5/102 \times 17.4 = 12$ would be expected in these 70.5 non-carriers. Of the total 27 deaths from malignant neoplasms actually found, $27 - 12 = 15$ could be attributed to the heterozygous carriers of the FA gene, and the risk that an FA heterozygote will die from a malignant neoplasm is thus $15/31.5 = 0.48$. At the extremes of the 95% confidence limits for the expected number of cancer deaths

and for the estimated number of FA heterozygotes in the sample, the same computation produces a lower estimate of 0.295 and an upper estimate of 0.84.

Table 3 Distribution of Deaths by Prior Probability of FA Heterozygosity

Probability of heterozygosity (p)	Number of deaths (N_p)	Estimated number of FA heterozygotes who died (column 1 $\times$ column 2)
1.0	2	2
0.67	1	0.67
0.5	26	13.0
0.25	54	13.5
0.125	19	2.375
Total 102		$N_h = 31.545 \pm 4.35$ ($\pm$ s.d.)* = the estimated number of FA heterozygotes in the 102 deaths.

* This standard deviation is computed as

$$\left[\sum_{p=0.1}^1 N_p \cdot p \cdot (1-p) \right]^{1/2}$$

The proportion of cancer deaths among all deaths has grown steadily over the years. In 1940, 0.12 of all deaths in the white population of the United States were due to a malignant neoplasm, in 1950, 0.15 and in 1960, 0.16¹⁵. The estimate of 0.48 for the risk of an FA heterozygote dying from a malignant neoplasm is thus about three times as great as normal, while the lower and higher estimates represent twice or five times normal, respectively.

Prevalence of FA Heterozygotes

There are no precise estimates of the incidence of FA homozygotes or of the frequency of the FA gene. A minimal estimate can be derived from the number of FA cases born in New York State from 1956 until 1967. Taking only the families I know, twelve definite cases have been born in New York State in that period among a total of 4.185×10^6 live births, providing an estimated birth incidence of 1 in 3.48×10^5 . If the normal and FA alleles follow the Hardy-Weinberg law, the expected heterozygote frequency is about 1 in 300. If the FA heterozygotes are, as the present data suggest, about three times as likely as non-carriers to die from a malignant neoplasm, then FA heterozygotes could comprise 1% of all patients dying of cancer or leukaemia.

For particular malignancies, the proportion of FA heterozygotes among all patients with that neoplasm may be considerably higher than this. For example, assuming that all three leukaemia deaths in the present study were in FA heterozygotes, then, using the estimate of 31.5 FA heterozygotes in the sample,

$$\frac{3}{31.5} = \frac{\text{estimated proportion of deaths from leukaemia}}{\text{among all deaths of FA heterozygotes.}}$$

If the FA heterozygotes are 1 in 300 in the population, then

$$\frac{3}{31.5} \cdot \frac{1}{300} = \frac{\text{leukaemia deaths in FA heterozygotes}}{\text{all deaths}}$$

$$0.00032 = \frac{\text{leukaemia deaths in FA heterozygotes}}{\text{all deaths}}$$

If 0.006 of all deaths are due to acute leukaemia (from recent mortality statistics), then

$$\frac{0.00032}{0.006} = 0.053$$

or about 1 in 20 is the estimated proportion of FA heterozygotes among all patients dying of acute leukaemia. This

approximate calculation demonstrates how important the FA gene may be in predisposing to particular malignant neoplasms.

Other Syndromes

The method of data collection and analysis used here can be applied to other autosomal recessive syndromes associated with cancer and leukaemia. If all or most of the genes which are neoplasia-predisposing in the rare homozygotes also have a significant effect in the heterozygous carrier, perhaps one of this set of genes is present in 5 or 10% of all cancer and leukaemia patients. Because there is no direct way at present to identify the heterozygous carriers of the neoplasia-predisposing genes, they would go unrecognized even when a malignant neoplasm occurs. A better understanding of the action of carcinogenic chemicals and oncogenic viruses in man may require isolating the genetic factors important in common human neoplasms.

The metabolic error associated with FA can be investigated in skin fibroblast cultures because cultures derived from FA homozygotes differ from normal in their "spontaneous" chromosome aberration frequency and in their susceptibility to transformation by the oncogenic virus SV40 (ref. 11). Knowledge of the immediate biochemical effects of the FA gene and, perhaps, of the role of this gene in predisposing to malignancy, might thus be conveniently derived from studying the easily identifiable homozygote, and applied to the clinically more important heterozygote. If this led to a specific test for the FA heterozygote, the neoplasia-predisposing effects of the gene could be measured precisely by comparing the heterozygote frequency in groups of cancer and leukaemia patients with that in the general population. Rare recessive syndromes, such as Fanconi's anaemia, may be of great value in understanding the origin of clinically common and important neoplasms.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Free Amino-acids dissolved in North Atlantic Ocean Waters

THE "dissolved" fraction of the organic matter in seawater is conventionally defined as that which has passed through a 0.45 μm filter¹ (which excludes zooplankton and phytoplankton and most marine bacteria, but not colloidal particles). An acceptable figure² for the mean concentration of this material in surface water is 1.2 mgC l.⁻¹ and in deeper water is 0.5 mgC l.⁻¹. Attention was focused on the amino-acids in seawater because, although they are the most difficult chemical problem because of their polar nature, the free amino-acid "pool" in the oceans is probably one of the best indicators of the state and rate of biogeochemical interactions³.

Table 1 Station Locations in the North Atlantic Ocean

Dates	Cruise	Station No	Latitude	Longitude	Depth to bottom (m)
March 10, 1966	—	16	44° 38' N	63° 36' W	12
April 23 to May 12, 1966	0266	14	59° 58' N	41° 35' W	1,900
		15	59° 17' N	40° 26' W	2,780
March 6–8, 1967	0267	11	44° 05' N	63° 05' W	180
		12	42° 40' N	61° 55' W	250
		13	42° 00' N	61° 10' W	4,100
April 5 to May 8, 1967	0767	2	41° 35' N	15° 25' W	5,490
		3	36° 23' N	8° 58' W	2,611
		4	38° 19' N	15° 08' W	5,363
		5	38° 34' N	19° 15' W	5,326
		6	38° 19' N	37° 58' W	3,922
		9	38° 18' N	54° 57' W	5,436
		10	38° 25' N	60° 11' W	5,074

Seawater was collected in 28 l. PVC Niskin bottles at fifteen stations in the North Atlantic during three cruises in the same season of 2 successive years (Table 1). It was filtered by gravity through precombusted silver membrane filters ('Flotronics', pore size 0.45 μm), acidified to pH 2 and immediately frozen. The samples remained deep-frozen (for up to 2 yr) until they were lyophilized. The dried salts were then extracted with aqueous acidified ethanol (80%, pH 1.3). The extract was evaporated to dryness, and the residue taken up in double-distilled water and de-ionized on a cation-exchange column ('Dowex 50W- $\times$ 12', 100 to 200 mesh, H⁺ form). The eluate was dried, trimethylsilyl derivatives were prepared and the amino-acid content was determined by gas chromatography. An internal standard, *p*-methyl phenylalanine, was added to all seawater samples after collection. Samples in which the recovery rate of standard was less than 65% were rejected. Standard solutions of amino-acids (as used for the calibration of Beckman amino-acid analysers) were used to evaluate the precision and

accuracy of the determination of amino-acids. Duplicates agreed with a mean coefficient of variation of 1.5% and the mean relative error from the stated composition of the standard was 4.8%, with relative errors of individual amino-acids ranging from 0.8% to 11% (Ala) (ref. 4).

The results for all samples are given in Table 2. No stable trimethylsilyl derivatives could be made for Arg, Cys Cys and His. The most frequent were the monoamino-monocarboxylic (neutral) amino-acids. These, with the exception of Ile, were also the most evenly distributed through the water column. Both the acidic amino-acids (Asp and Glu) and the one basic amino-acid (Lys) were present only in small quantities, and both Glu and Lys, but not Asp, were spread unevenly through the water column. In the only directly comparable study⁵ of the variation in occurrence of sixteen dissolved free amino-acids at fifteen depths to 3,600 m, the basic and the acidic amino-acids also had the lowest frequencies of occurrence, although differences were small, and the conclusion was therefore that "the free amino-acid spectrum of the Pacific Ocean off California is surprisingly uniform and exhibits no significant qualitative changes throughout most of the water column". My results indicate the opposite, namely that the free amino-acid spectrum of waters in the North Atlantic Ocean is non-uniform and varies qualitatively and significantly with depth. (My samples were, however, taken from more widespread and generally deeper stations than those of the previous study.) Significant differences in concentration through the water column were shown by the following: Ala was more concentrated in the surface and central water (0–800 m) of the North Atlantic than in the bottom water (3,500–5,400 m); Leu, more in the surface and central water than in the deep water (800–3,500 m); and Phe, more in the surface and central water than in both the deep and the bottom water.

Further comparison with Degens, Reuter and Shaw's results⁵ shows that: Ala, Gly, Ser, Thr, Val and Leu concentrations were relatively high in both analyses; Lys, Tyr, Glu and Asp were low in both; Gly, Lys and Asp concentrations were highly variable in both; and Ala, Phe and Thr were relatively invariant in both. The findings of Chau and Riley⁶ are similar to data presented here, in that neither Orn nor His was detected, but Met was found. The most abundant amino-acids—Ala, Gly, Ser, Thr and Val—were the same in all three investigations. The routinely high occurrence of Ala, Gly, Ser and Thr in other aqueous environments should be noted⁷.

Individual sample amino-acid totals ranged from 6 to 47 $\mu\text{g l.}^{-1}$ with a mean of 22 $\mu\text{g l.}^{-1}$, but the surface and central water totals were consistently above this mean. The difference between the mean concentration in the surface and central water and the bottom water was significant at the 0.1% level, but the difference between the deep water and the bottom water was not significant. The high surface and subsurface totals reflect the appreciable contributions of Ala, Leu + Ile, and so on, which are greatly reduced below about 800 m, whereas the contributions of Ser + Thr and Asp + Glu are increased.

Table 2 Concentration in nmol l.⁻¹ of Free Amino-acids dissolved in North Atlantic Ocean Waters

Cruise	Station	Depth (m)	Ala	Gly	Val	Leu	Ile	Pro	Ser	Thr	Hyp	Asp	Met	Glu	Phe	Lys	Tyr	Sample totals		
																		µg l. ⁻¹	µgC l. ⁻¹	µgN l. ⁻¹
0266	16	0	155	—	33	39	22	21	+	+	+	+	+	+	36	56	28	47.1	24.1	6.2
	14	92	73	35	14	13	8	8	76	—	7	—	—	55	—	7	+	32.3	13.2	4.2
	15	2,188	176	26	23	39	10	3	—	3	17	—	—	45	28	17	21	47.0	22.4	5.9
0267	11	50	22	—	+	9	7	—	+	+	—	2	+	73	+	2	—	15.2	6.5	1.6
		100	132	42	98	22	8	?	21	28	4	—	6	—	4	+	?	38.0	17.0	5.1
	12	50	19	104	31	30	9	?	22	22	—	—	—	?	22	?	8	28.1	12.9	3.7
0767		100	130	173	41	7	—	—	—	18	4	—	17	—	14	—	—	37.9	15.5	5.7
	13	100	179	39	39	9	—	—	—	37	10	—	8	—	19	—	—	34.6	15.2	4.8
	2	5,140	43	35	2	41	—	4	—	19	—	—	28	—	31	5	6	25.6	12.5	3.0
		5,360	146	—	10	20	—	—	—	—	—	—	1	—	1	+	+	17.0	7.4	2.5
	3	280	25	79	26	38	4	3	54	76	1	—	+	—	21	—	+	35.3	15.5	4.6
		840	21	6	44	5	+	—	—	69	18	—	+	—	12	—	?	20.8	9.7	2.5
		1,760	46	+	28	40	+	+	—	9	3	—	25	—	20	27	—	24.8	12.3	3.1
		2,500	130	—	23	15	—	—	—	17	—	—	—	—	—	—	+	18.2	7.9	2.6
	4	980	27	+	15	13	—	?	—	48	—	+	+	+	+	—	—	11.7	5.2	1.4
		2,960	23	+	34	20	—	?	26	—	—	—	—	—	—	—	—	11.3	5.2	1.4
		4,450	37	+	24	26	?	2	—	47	—	16	+	—	6	—	—	18.4	8.4	2.2
		5,220	41	—	1	12	—	?	+	19	—	7	10	—	8	—	—	11.3	5.1	1.4
	5	960	53	—	15	5	+	—	—	35	1	2	—	—	4	—	—	12.2	5.4	1.6
		2,882	161	—	31	21	—	—	—	+	—	+	5	5	5	—	—	22.9	10.3	3.2
		4,340	81	—	43	11	5	—	46	18	5	+	?	—	11	—	3	24.5	11.1	3.1
		5,180	28	—	46	18	—	—	—	+	+	—	6	—	+	—	—	11.1	5.4	1.4
	6	985	46	34	8	40	—	—	49	—	—	—	—	—	—	—	—	17.9	7.6	2.5
		1,840	176	—	—	13	—	—	10	—	—	—	1	—	—	—	—	18.6	7.7	2.8
		2,970	35	—	3	+	?	?	171	—	—	—	—	—	—	—	—	21.5	7.6	2.9
	9	1,000	115	?	?	33	9	?	—	—	—	—	?	?	4	—	—	16.4	7.6	2.3
		3,000	34	—	+	7	19	—	—	—	—	—	—	—	—	—	—	6.4	3.1	0.8
		4,500	66	+	34	15	19	—	—	—	—	+	+	+	8	—	7	16.7	8.4	2.1
	10	150	157	—	10	14	6	—	+	—	—	—	—	—	12	—	19	23.1	11.0	3.0
		990	39	10	1	1	2	1	—	42	—	—	—	68	18	—	—	22.7	10.0	2.5
		1,900	141	1	2	—	—	—	—	—	—	—	—	—	—	—	—	12.8	5.2	2.0
		3,800	23	—	9	10	—	5	1	—	—	1	—	—	1	4	14	8.2	4.2	1.0
		4,800	19	—	—	11	+	+	+	+	—	—	+	—	+	1	13	5.6	2.9	0.6

The samples are listed in the order in which they were collected and analysed. The amino-acids are listed in order of elution from the gas chromatograph. —, Amino-acid absent; ?, presence questionable; +, present but not quantifiable.

Variations in the total quantity with depth correspond usually with variations in quantity of the most frequently occurring amino-acids.

The contribution of the dissolved free amino-acids to the total dissolved organic carbon ranged from 3 to 24 µgC l.⁻¹, with a mean of 10 µgC l.⁻¹, which is about 2% of the total dissolved organic carbon. The contribution to the dissolved organic nitrogen ranged from 0.6 to 6 µgN l.⁻¹ with a mean of 2.8 µgN l.⁻¹, which could be as much as 4% of the total amount of dissolved organic nitrogen, and could constitute up to 14% of the "ammonia" measured by one of the oxidation methods⁸.

My results show that although the concentration of dissolved organic carbon below a depth of 400 m may be relatively invariant⁹, compounds comprising this total can vary greatly. I infer a dynamic, rather than a static, role for this fraction of the dissolved organic matter, with interactions taking place within the limits of precision of the present analytical techniques for dissolved organic carbon.

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Internal Cylindrical Reflexion in a Cubic Crystal

WE wish to report an optical effect, in which a point source of light viewed through a crystal of potassium tantalate-niobate (KTN) was spread out into a ring, which passed through the source. The explanation of this effect is clear in principle but puzzling in detail.

As part of a study of electro-optic materials, we have grown several crystals of KTN using the method described by Bonner¹. This perovskite material has cubic symmetry above its Curie temperature, belonging to the crystal class m3m. The crystals were pulled from the melt in the (001) direction, with rotation about this axis. The optical effect was first observed in the central region of one of these crystals. This region (with dimensions about 1 cm³) was cut from the crystal and polished on two surfaces normal to the growth direction. Although this specimen had several imperfections, the rest of the crystal had been accepted for use in making optical modulators. The Curie temperature of the complete crystal was 9° C and as the observations were made near 20° C the crystal was certainly in the cubic phase. The ratio of tantalate to niobate indicated by this Curie temperature is 62:38.

Looking towards the Sun through the crystals, we observed a sharp ring, slightly bluish in colour. The orientation and angular subtense of the ring depended on the direction and magnitude of the tilt of the crystal (001) axis away from the direction of the source. The effect varied in luminance as the eye was moved relative to the exit face of the crystal. Viewing through a prism suggested that there was no strong wave-length dependence. Also inspection through polaroid showed that the effect was not noticeably polarization sensitive. We found it extremely easy to photograph the ring using a helium-neon laser and a camera set for infinity (Fig. 1). The diameter of the ring shown subtended an angle of 10° at the crystal, resulting from a crystal tilt of 5°. The thickness of the ring is thus equivalent to 0.2°.

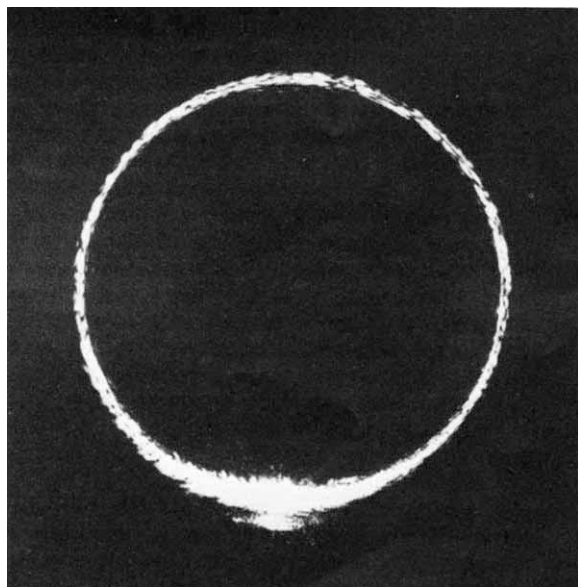


Fig. 1 Ring effect produced by laser beam incident at 5° to the crystal specimen.

In view of these properties it seemed likely that the effect depended on reflexion within the crystal; it resembled the reflexion of light from a thin fibre or a polished wire^{2,3}. Because the effect was observable throughout the specimen we postulated that the crystal contained a number of rod-like imperfections oriented along the growth direction and uniformly distributed throughout the specimen. In Fig. 2 we indicate how a crystal tilt of θ may produce an image of the source displaced by 2θ , as a result of reflexion at a rod-like imperfection. The effect of rotating the crystal about the axis of the rod by $\pm 90^\circ$ will clearly be to produce a cone of images as seen by the eye. Attempts were made to identify the reflecting elements involved, using a polarizing microscope at magnifications between 45 and 1,500. A few thread-like imperfections extending through the depth of the crystal directly below the seed crystal were noticed, which seemed to be refractive index inhomogeneities in the form of rods, rather than hollow tubes, because the ends did not appear to intersect the crystal surface in holes. Such inhomogeneities are probably caused by bubbles of flux trapped beneath the seed travelling through the crystal as it is pulled. The concentration of these defects did not seem great enough to account for the intensity of the ring structure observed, there being fewer than a hundred

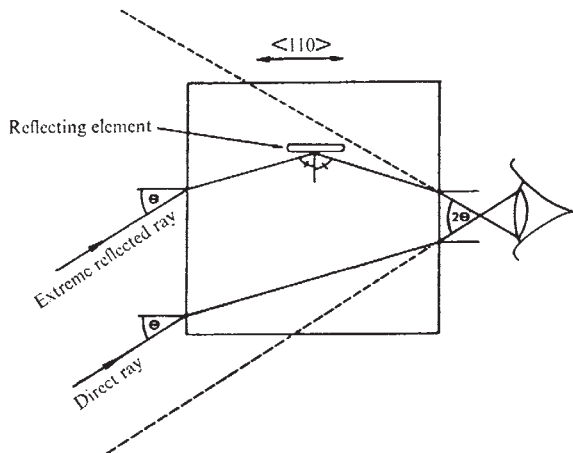


Fig. 2 Diagrammatic representation of the effect of tilt on the crystal.

threads in the whole crystal. To obtain more convincing evidence we decided to polish two more faces on the specimen in order to view at right angles to the growth direction. In this way we expected that any small imperfections would have a greater dimension along the growth direction, so that they could be observed more easily. In addition, it was predicted that in place of the ring phenomenon a bar image would be observed when viewing in this direction.

At first we did not see many randomly distributed, rod-like imperfections when examining the crystal in the (010) direction, only a few of the large rods being visible. A lamellar pattern was visible, however, in the plane perpendicular to the growth direction when the crystal was placed between crossed polarizers¹ (Fig. 3). The striations have a periodicity of about 0.04 mm and are observable in most of our specimens, with or without crossed polarizers, in this direction. When a point source was viewed in the new direction a bar image was clearly visible. In certain areas two bars inclined at an angle were observable, apparently because part of the crystal had striations inclined at about 40° to the remainder (Fig. 4). It is doubtful whether the existence of these particular lamella could account for the ring effect as they lie in a direction perpendicular to that of growth. Because of the absence of noticeable rods we were surprised to find that the ring structure was also visible, although with reduced luminance, when viewing in the (010) direction. For some positions of the eye a double ring pattern was visible, presumably associated with the existence of different patterns of striations in the two parts of the crystal. Neither striae nor a double ring effect were seen in the growth direction, but this might be because the inclined striations would reflect light at 80° relative to the (001) direction and such rays would be internally reflected.

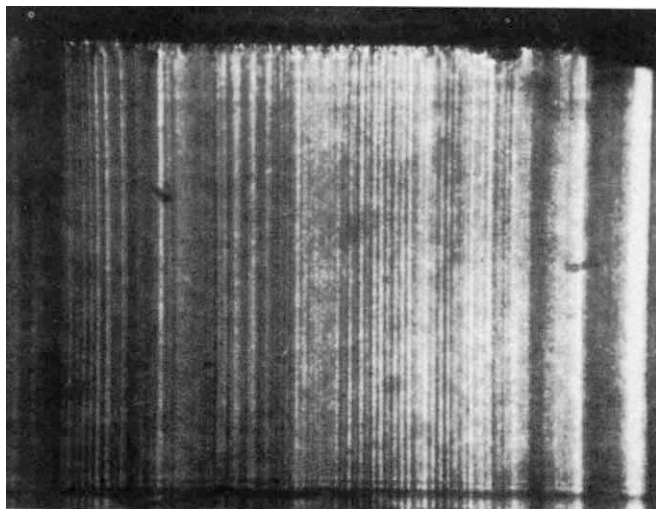


Fig. 3 Lamellar pattern normal to the growth direction.

As a result of this second experiment we abandoned the hypothesis that the effect was caused by cylindrical symmetry about the growth direction and concluded that the controlling symmetry is that of the cubic lattice, the imperfections being oriented along the axes of the cube. The ring is more pronounced in the growth direction but this might be because of the additional reflexions from the few rods observed. We are still in doubt about the actual nature of the fine reflecting elements. The effect may be similar to that observed with some specimens of fascicular gypsum², although a strong dependence on the polarization of the incident light would be expected in this case. It is more likely that rod-like inhomogeneities (bubble tracks?) in the crystal cause Rayleigh-Gans scattering³. The sharpness of the ring indicates that if several reflectors are involved they are aligned to within 0.1° . Some polarization-dependence would be anticipated in this case also, although

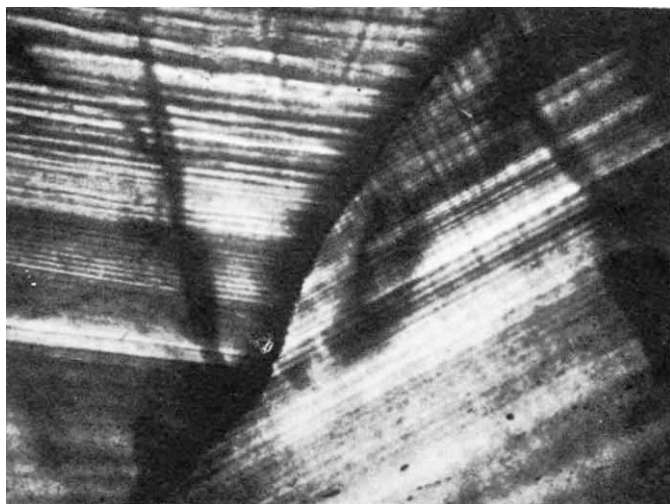


Fig. 4 Two lamellar patterns inclined at 40°.

more refined methods than visual examination would be needed in order to detect it. Our observation of rings in the (010) direction suggests that some rods are aligned perpendicular to the growth direction: it is not easy to account for the formation of these rods.

It would be interesting to know whether similar effects are observed with other types of cubic crystal. Certainly the outer parts of the same KTN crystal show the effect faintly in both directions, although they are otherwise much superior optically. Also, if the effect is linked to the lattice symmetry rather than the growth direction, one is led to suggest that different, more complicated ring structures may be observable in crystals with symmetries other than cubic, provided they have a sufficient concentration of imperfections. In view of the perfect external shape of the KTN crystal we checked whether the lattice orientations on either side of the boundary shown in Fig. 4 were different, by recording Laué X-ray back reflexion patterns from the two surfaces normal to the growth direction: the patterns were identical, showing that the lamellar pattern need not exhibit all the symmetry of the crystal.

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Auger Electron Spectroscopy of Graphite Fibre Surfaces

THE atomic composition of the external surface of graphite fibres has recently been investigated by the application of high energy photoelectron spectroscopy¹. Because the structural characteristics of graphite/epoxy composites are usually evaluated by measuring the short beam shear strength (a measure of interlaminar shear), it is important to be able to correlate this with the bonding between the fibres and the matrix material. High energy photoelectron spectroscopy

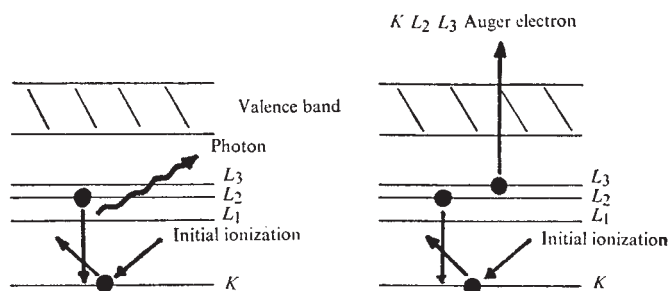


Fig. 1 Processes leading to X-ray and Auger electron emission.

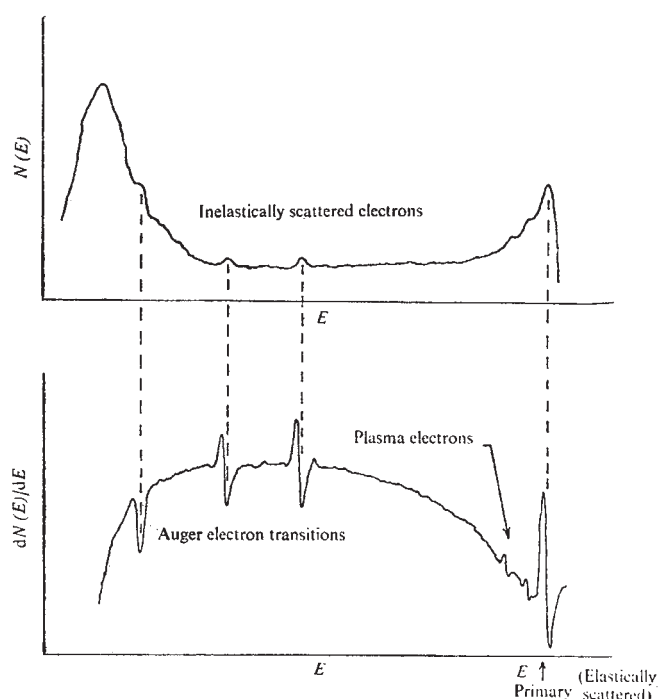


Fig. 2 The energy distribution curve and its derivative.

involves measuring the binding energy of core electrons excited by low energy X-rays, and changes in the chemical environment in a region between 100 Å and 150 Å thick are reflected by a subsequent slight shift of the energy of the core electrons.

We present here an alternative method for surface chemical analysis, known as Auger electron spectroscopy. Laboratories everywhere are beginning to use this technique for a variety of surface studies²⁻⁷.

In the Auger process, an electron beam, incident on a solid surface, may ionize the uppermost atom by removing a tightly bound (such as a K level) core electron. The excited atom may return to its ground state when a more loosely bound electron (such as one in the L_2 level) falls to the K level. The atom will now have excess energy equal to $E_K - E_{L_2}$. This energy may be released by two processes. The first process, photon emission, is well known and forms the basic technique of X-ray fluorescence. The second process which may occur involves transferring the excess energy to another loosely bound electron (such as one in the L_3 level), ejecting it from the atom. These two competing processes are shown in Fig. 1. The KL_2L_3 Auger electron would have an energy (neglecting work function, surface and contact potentials) equal to $E_K - E_{L_1} - E_{L_3}$. The X-ray yield decreases sharply as the atomic number decreases, making the detection of Auger electrons a very good technique for identifying light elements with an atomic number less than 20 (although much heavier elements may also be detected).

Experimentally, the Auger electrons are detected by recording the derivative of the energy distribution ($dN(E)/dE$) of the electrons emitted, when a sample is bombarded by a

primary electron beam of approximately 1 mm diameter (1–3 keV, 10–50 μ A). The relationship between $N(E)$ and $dN(E)/dE$ is shown in Fig. 2, while Fig. 3 shows the electronic circuit used to obtain Auger electron spectra in a low energy electron diffraction (LEED) system which is kept at an ultra high vacuum (less than 10^{-9} torr)^{8–11}.

Because the mean escape depth for Auger electrons in silver (without significant energy loss) varies between 4 and 8 Å for energies of 72 and 362 eV, respectively¹², Auger electron spectroscopy is an ideal method for analysing the elements present on the graphite fibre surface (that is, on the first few atomic layers). The technique of high energy photoelectron spectroscopy examines a layer 100 to 150 Å in depth¹. Moreover, Auger electron spectroscopy is very sensitive; 0.02 monolayers of caesium on a silicon surface have been detected⁵ and routine analysis of surface elements from parts

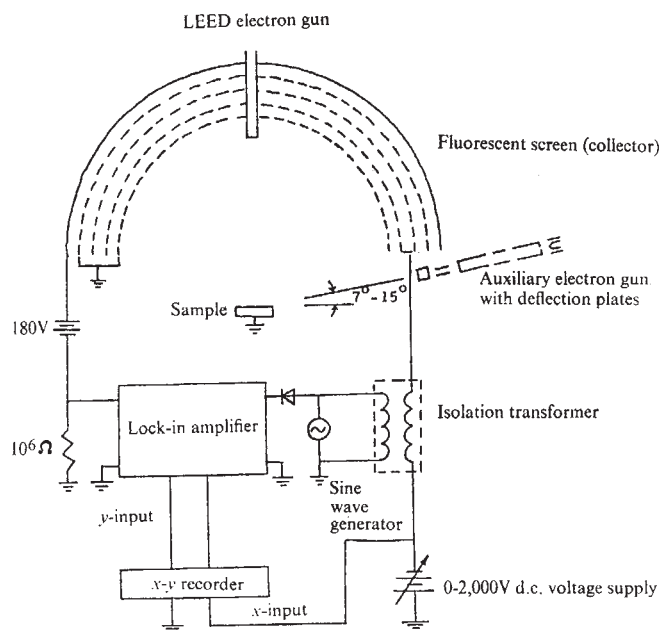


Fig. 3 Electronic circuit for obtaining Auger electron spectra in a LEED system.

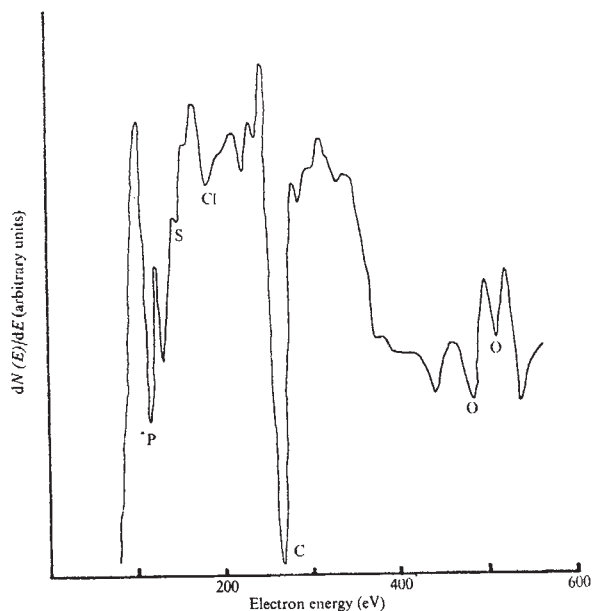


Fig. 4 Morganite II fibre, $E_p=1,450$ V, $I_p \sim 30$ μ A.

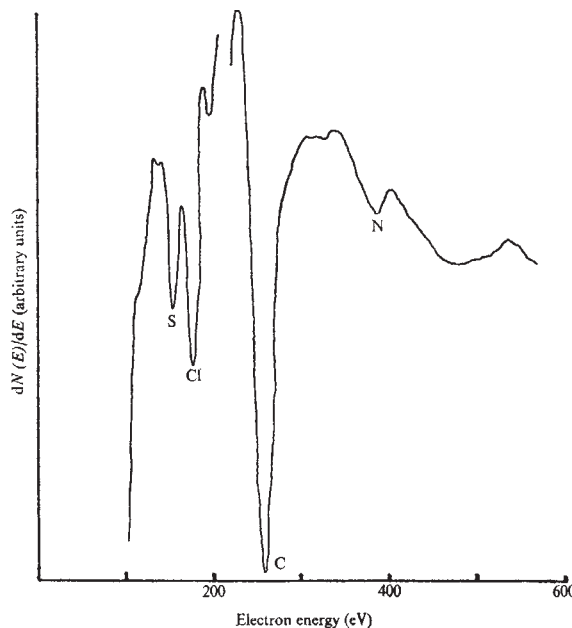


Fig. 5 Courtaulds HT-S fibre, $E_p=1,450$ V, $I_p \sim 30$ μ A.

per thousand to parts per million is possible when accurate procedures and techniques are used. Chemical shifts have been detected for carbon and carbon monoxide on molybdenum¹³, and the band structure of silicon has been investigated by analysis of the fine structure present in characteristic Auger spectrum¹⁴.

In a study of graphite/epoxy composites, we obtained the Auger electron spectra from two types of graphite fibres, Morganite II and Courtaulds HT-S. When made into a composite, these have an average short beam shear strength of 12,000 and 8,500 pounds/inch², respectively. The samples were irradiated with a primary electron beam of 1,450 eV, 30 μ A. These fibres were in an "as-received" condition because we were interested in the elements present in the interfacial graphite/epoxy bond as they would exist in the finished composite structure. The results are shown in Figs. 4 and 5. In the high energy photoelectron spectroscopy study¹, the effects of heating in an inert atmosphere and oxidation were examined. This was not done in the study described here. However, the use of Auger electron spectroscopy in a more detailed study should be very helpful in furthering an understanding of the graphite/epoxy bond and improving the inter-laminar shear in these composites.

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Atmospheric Fluorine Compounds as Indicators of Air Movements

GASEOUS fluorine compounds are supposed not to occur naturally in the atmosphere. Volatile fluorine compounds would not be expected to result from chemical equilibria between fluorine compounds on the surface of the Earth, and it is improbable that biological systems contribute significant quantities of organic fluorine compounds.

It is, however, estimated that about 10^6 tons of fluorine compounds are released each year into the atmosphere, and these include halomethanes from aerosol dispensers, fire extinguishers, refrigerant fluids and anaesthetics, and sulphur hexafluoride from electrical equipment. By contrast with these stable compounds, reactive compounds such as hydrogen fluoride, which are also industrial products, are rapidly scavenged from the atmosphere by physical and chemical processes.

The presence of stable sulphur and carbon fluorides in the atmosphere is not in any sense a hazard, and their existence has only been detected by the very sensitive technique of gas phase electron absorption. The fluorides are, however, of special interest because they enter the atmosphere only from industrial and domestic sources, whereas other gaseous industrial emissions are also natural products; their distribution in the atmosphere can therefore be a useful indicator of air movements and wind directions.

Table 1 Observations at Adrigole, Co. Cork, Ireland ($51^\circ 40' \text{ N}$, $09^\circ 45' \text{ W}$)

Wind heading	Concentration by volume		Turbidity
	CCl_3F	SF_6	
$45^\circ\text{--}135^\circ$	1.0×10^{-11}	2.9×10^{-14}	0.03
	(4)	(3)	(7)
$225^\circ\text{--}315^\circ$	1.9×10^{-10}	1.2×10^{-13}	0.19
	(3)	(3)	(2)

The number of observations is shown in parentheses.

We report in this communication preliminary measurements of the atmospheric concentrations of sulphur hexafluoride and trichlorofluoromethane in south-west Ireland during July and August 1970. Analyses were made using a gas chromatograph with an electron capture detector. Experimental conditions were arranged so that ionization in the detector was complete¹ and under these circumstances errors are only likely to be caused by loss of sample during collection and chromatography; for example, by irreversible adsorption. Calibration of the system showed that such losses probably did not exceed 30%. Our results are shown in Table 1; the atmospheric turbidity was measured with a 'Volz' sun photometer using the technique described by Flowers, McCormick and Kurfis². Air arriving at the monitoring site from a north-westerly direction does not pass over any source of either CCl_3F or SF_6 and so the concentrations measured under these conditions can be regarded as representative of the northern hemisphere background. If the industrial outputs of these compounds are assumed constant at about 2×10^5 and 10^2 tons per year respectively, the atmospheric lifetimes would seem to be 1 yr and 4.6 years.

Although other halocarbons such as CF_2Cl_2 and perfluorocyclobutane are almost certainly present in the atmosphere, they were not observed in our preliminary experiments because of their relatively low rate of reaction with thermal electrons in the electron capture detector. An unknown electron absorbing compound was, however, always found to be present in the air at a concentration of about 3×10^{-11} by volume and, although its retention time in the chromatograph column suggested a boiling point of about 75°C , it has not been possible to identify it.

The high concentrations of CCl_3F and SF_6 associated with easterly winds from continental Europe and the appreciably

increased turbidity lend support to the proposal that these compounds can be used as indicators of air masses which have recently been polluted by industrial effluents.

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Reply to Cherry, Barnes and Fullman

My answer to the question raised by Cherry, Barnes and Fullman¹ about the structure I proposed for anomalous water² can only be "why not?" I agree that my structures are similar to the structures of ice 1h and 1c, but they are not just a scaled down version of them. I demonstrated how the structures could be built up from six-molecular rings and therefore one can see that it might be the way water molecules hold themselves together to form ice. I also indicated that the anomalous water could only be formed in the presence of foreign cations and was analogous to the structure of water in concentrated aqueous salt solutions. I do not see why the structures cannot be liquid under the condition described.

An obvious question to ask is how far out from the capillary wall these structures (or anomalous water) can be maintained without foreign cation impurities among the rings. The answer may be the chief reason for the difficulty in obtaining pure anomalous water and the questioning of its existence. I believed, when proposing the mechanism, that the oxygen ion in the water molecule was depolarized and became more ionic when it was surrounded symmetrically by four hydrogen ions (or cations) and the molecule as a whole was further polarized. The stretching of the O-H bond is therefore the transformation of covalent bonding to ionic bonding. It seems that the lone pair has a tendency to attach to a bare cation, as in the formation of complexes, and the experiments with anomalous water indicate that water molecules may take some cations with them when evaporating from salt solutions: this is disturbing if true.

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Fluid Orthodoxy?

A RECENT article¹ by the molecular physics correspondent of *Nature* began: "If there is an orthodoxy in the theory of liquids . . . it has little place for notions which depart very far from the familiar, well founded and regrettably somewhat sterile formalism of Gibbsian statistical mechanics". We suggest that the problem with the statistical mechanics of Gibbs and Boltzmann is more closely related to frigidity than sterility; the response is difficult to evoke, but once evoked is usually fruitful. It is surely unfair to brand as sterile a septuagenarian formalism which gives birth to a complete description of solids², liquids^{3,4} and gases⁵ and of the phase equilibria between them⁶. No doubt the impression of sterility arose from the long gestation period which was necessary

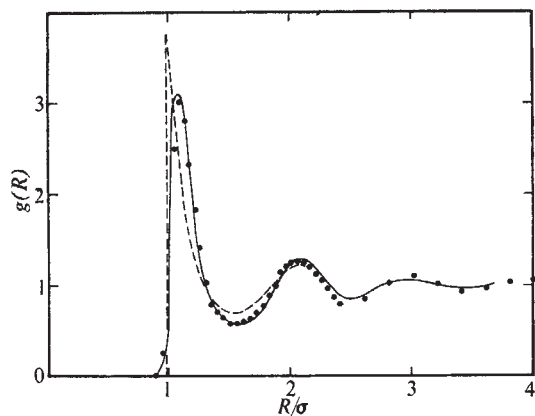


Fig. 1 Radial distribution function for liquid with 6:12 interactions (depth ϵ , diameter σ) for $T^* = kT/\epsilon = 0.72$, $\rho^* = N\sigma^3/V = 0.85$. Dotted curve, zero order hard sphere distribution function; solid curve, first order distribution function. The circles are molecular dynamics results of Verlet²⁰.

before modern computers permitted the explicit demonstration of the full richness of content of statistical mechanics.

The same article suggests that the "random geometric" approach to liquids, ably propounded by Bernal and his co-workers⁷⁻⁹, is unexplored because it is unorthodox. In fact the importance of random packing of hard cores in determining the properties of liquids has been recognized since the time of van der Waals¹⁰, who incorporated this concept in his well known equation of state. More recently the ideas of van der Waals have been developed by Longuet-Higgins and Widom¹¹ and by Guggenheim¹², and have been given a rigorous basis in statistical mechanics by the development of perturbation theories¹³⁻¹⁹ using the hard sphere fluid as the unperturbed system. The latter approach has led to a quantitatively satisfactory and qualitatively appealing theory of liquids; the properties of a fluid are determined by expansion in powers of parameters representing the strength of the attractive forces and the softness of the repulsive potential. In zero order the structure of the fluid is determined by the random packing of hard spheres (given a precise definition by the statistical mechanics of Gibbs); in first order the structure is somewhat modified by the attractive forces and the softness of the repulsive potential, and it turns out that higher order terms are negligible. Both the zero order and the first order radial distribution functions are shown in Fig. 1. The first order curve is in excellent agreement with the "quasi experimental" results calculated by the method of molecular dynamics by Verlet²⁰. (Actually the method of molecular dynamics rests ultimately on the ideas of Boltzmann, but comparison with a real experiment would lead to identical conclusions.) The calculation of these curves²¹ relied on detailed knowledge of the relevant aspects of the "statistical geometry" of hard spheres derived from Monte Carlo computer calculations¹⁹⁻²¹ using the statistical mechanics of Gibbs. There is also an integral equation theory²² which gives an excellent approximation to the properties of the hard sphere fluid.

There are two obstacles to the fruitful implementation of Bernal's polyhedron statistics approach to statistical geometry in the theory of liquids. The first is that "random packing" is not a precisely defined concept until one specifies some ensemble (Gibbs?) or population from which one is choosing randomly—this point was made clearly by Bernal⁸ in connexion with the reproducibility of measures. The second is that no one has yet found a fully satisfactory way of using the polyhedron statistics description to make progress. It may be that the route lies in homology theory¹⁹, but homology theory may well be as discouraging to a molecular physics correspondent as configuration integrals. It is perhaps these difficulties that have prevented progress rather than an unyielding orthodoxy; if there is an orthodoxy in the theory

of liquids, one surely expects it to be fluid and eclectic rather than rigid.

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OUR molecular physics correspondent writes: On the whole, I am unrepentant. What worries me in the above is the pious implication that we should be content with one particular mathematical method said, with rather slim justification, to give birth to a "complete description" (no less) of the states of matter and their phase equilibria. Precisely what sense are the words "complete" and "description" supposed to carry in this context? Would Barker and Henderson accept, at least for the purposes of my article, that there is an element of difference between a "description" of the states of matter and an "understanding" of them, just as it is by "predictive" rather than "descriptive" power that any theory must ultimately stand or fall, even in a field as difficult as the theory of liquids?

The use of computers for molecular dynamic calculations has undoubtedly revitalized the classical theory, but the effect of this will be less telling if it leads to the neglect or undervaluation of alternative approaches, particularly those which, for want of a label, might be called information theoretic in standpoint. And, in speaking of undervaluation, I would specifically include the tendency to see a radical innovation such as Bernal's as just another formulation of received ideas. In the end this attitude, fluid and eclectic perhaps, could be as inhibiting to original work as the outright intolerance which so often went with orthodoxy in Gibbs's day.

My prediction, from a relatively detached position, is that information theoretical methods will prosper in the long run, that their advance will be only marginally dependent on computer technology and that the heterodox views of the Birkbeck school will be a historically significant, though probably not a central, feature of this trend. Whatever else, Bernal's theory of liquids is imaginative—in several flavours of the word—and, pending final justice, should be welcomed as such.

BIOLOGICAL SCIENCES

Adrenal Tyrosine Hydroxylase and Dopamine β -Hydroxylase in Spontaneously Hypertensive Rats

SPONTANEOUSLY hypertensive (SH) rats, a Wistar strain¹, are extremely useful for screening antihypertensive agents which inhibit the biosynthesis of catecholamine hormones. We describe here a pronounced hypotensive action of the inhibitors of tyrosine hydroxylase and dopamine β -hydroxylase on SH rats and an increase in tyrosine hydroxylase and dopamine β -hydroxylase activities in the adrenal glands.

The rats were 4 months old, with blood pressures between 160 and 200 mm Hg. Normal Wistar rats of the same age raised in the same conditions were used as controls.

The hypotensive effects of two newly described inhibitors of tyrosine hydroxylase and dopamine β -hydroxylase on SH rats are shown in Table 1. Oudenone is a new microbial product² which inhibits tyrosine hydroxylase *in vitro* and *in vivo* and reduces the concentrations of tissue catecholamines. Fusaric acid (5-butylpicolinic acid), an antibiotic produced by a fungus, has been shown³ to be a potent hypotensive agent and a potent inhibitor of dopamine β -hydroxylase, inhibiting adrenal dopamine β -hydroxylase activity *in vitro* and reducing the concentrations of tissue catecholamines⁴. Both oudenone and fusaric acid have marked hypotensive effects on SH rats (Table 1) and normotensive Wistar rats, although the former are significantly more sensitive.

To investigate the high susceptibility of SH rats to the inhibitors of tyrosine hydroxylase and dopamine β -hydroxylase, the activities of the enzymes in the organs of SH rats were examined. Rats were decapitated and brains, hearts and adrenals were quickly removed. The brain stem was isolated and the organs were stored frozen on dry ice. Tyrosine hydroxylase was partially purified by ammonium sulphate fractionation from the soluble fraction of the tissues for the determination of the activity. The enzyme activity was determined by the formation of ¹⁴C-dihydroxyphenylalanine (dopa) from L-¹⁴C-tyrosine⁵. Dopamine β -hydroxylase was assayed, after solubilization and partial separation of the enzyme from the particle fraction of the adrenal glands^{6,7}, as described before⁸. These purification procedures of tyrosine hydroxylase

and dopamine β -hydroxylase permit the determination of the enzyme activities which are proportional to the amount of the enzymes.

Table 2 Activities of Tyrosine Hydroxylase and Dopamine β -Hydroxylase in the Adrenals and Brain Stem of Spontaneously Hypertensive Rats

Enzyme activity	Normal Wistar rats	SH rats
Tyrosine hydroxylase		
Adrenals		
nmol/min/adrenals $\pm$ s.e.m.	1.00 $\pm$ 0.10	1.99 $\pm$ 0.23 *
nmol/min/mg protein $\pm$ s.e.m.	2.06 $\pm$ 0.18	3.67 $\pm$ 0.29 *
Brain stem		
nmol/min/mg protein $\pm$ s.e.m.	0.244 $\pm$ 0.025	0.241 $\pm$ 0.026
Dopamine β -hydroxylase		
Adrenals		
nmol/min/adrenals $\pm$ s.e.m.	0.75 $\pm$ 0.11	1.59 $\pm$ 0.13 *
nmol/min/mg protein $\pm$ s.e.m.	0.57 $\pm$ 0.02	0.87 $\pm$ 0.09 *

* $P < 0.01$.

Eleven animals were used for measuring tyrosine hydroxylase activity; eight were used for measuring dopamine β -hydroxylase activity. The results were averaged.

The activities of tyrosine hydroxylase and dopamine β -hydroxylase in the organs of SH rats are shown in Table 2. Tyrosine hydroxylase activities in the adrenal glands of SH rats were significantly higher than those of normotensive Wistar rats. There was a two-fold increase in the enzyme activity. The increase in tyrosine hydroxylase activity in the adrenal glands of SH rats may have been caused by an increase in the amount of the enzyme. An increase in tyrosine hydroxylase activity in the adrenals has been reported before⁹. The results are consistent with the report of Ozaki¹⁰ that the concentration of noradrenaline in the adrenal glands of SH rats doubles 4–6 months after birth and that α -methyl-*p*-tyrosine, a tyrosine hydroxylase inhibitor, has a marked hypotensive effect on SH rats. Ozaki¹¹ also found a slight increase of tyrosine hydroxylase activity in the adrenal homogenates of SH rats. In contrast, the level of tyrosine hydroxylase activities in the brain stem of SH rats was similar to that of normotensive Wistar rats (Table 2). We also found that tyrosine hydroxylase activity in the heart of SH rats remains similar to that of normotensive Wistar rats.

Table 1 Effect of Oudenone (a Tyrosine Hydroxylase Inhibitor) and Fusaric Acid (a Dopamine β -Hydroxylase Inhibitor) on Blood Pressure of Spontaneously Hypertensive Rats

Dose (per os, mg/kg/day)	Blood pressure (mm Hg); time after dosing (h)							
	0	First dosing		Second dosing		Third dosing		
		6	24	6	24	6	24	72
Oudenone								
25	170	167	120	110	100	93	97	120
	167	147	150	130	113	120	110	123
12.5	200	177	167	160	140	150	130	150
	160	170	160	160	140	153	130	100
6.25	177	177	177	150	150	137	120	127
	160	163	127	130	127	130	117	170
3.1	160	140	150	157	103	143	100	107
	170	167	120	150	140	133	147	147
Fusaric acid								
40	187	103	127	110	117	103	120	133
	170	97	123	100	123	103	117	140
20	217	123	147	117	127	117	150	150
	170	100	120	100	127	90	120	140
10	197	120	137	120	110	123	130	140
	170	110	130	113	123	117	133	147
5	177	130	160	120	127	123	130	153
	170	113	153	120	130	127	140	180

Two rats were used in each experiment. The value of blood pressure is the average of three determinations.

Dopamine β -hydroxylase activities in the adrenal glands of SH rats were significantly higher than those of normal Wistar rats. The increase in dopamine β -hydroxylase activity in the adrenal glands of SH rats may be attributed to an increase in the amount of the enzyme as in the case of tyrosine hydroxylase.

The relationship between the marked hypotensive effect of the inhibitors of tyrosine hydroxylase and dopamine β -hydroxylase and the increase in the activities of both enzymes in the adrenal glands in the case of SH rats remains a subject for future investigation.

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Purity and Stability of Synthetic Peptides such as Angiotensins and Kinins

Now that synthetic biologically active peptides are becoming readily available, some biologists are using them without realizing that they are neither as stable nor as pure as sodium chloride.

The angiotensins are good examples. Many workers, knowing that angiotensin II sticks to glass, are careful to make up, store and use solutions in plastic containers or in siliconized glassware. We use polyethylene containers, but nevertheless dilute solutions of angiotensin II sometimes lose their potency. The inactivation is capricious and, when it occurs, may take 5 min or 5 h. It can also happen as the solution passes through the tubing of an infusion pump. The inactivation is presumably due to bacterial contamination, for it is prevented by an antibacterial procedure based on that of Leary¹. It consists of filling the tubing of the infusion pumps

and the polyethylene containers with a laboratory detergent such as 'Decon 75' overnight. After washing with distilled water for 5 min they are then washed with 0.01% solution (w/v) of the antiseptic chlorhexidine digluconate ('Hibitane', ICI) for 15 min. This is followed by further washing in distilled water for 5 min and sterile saline for 10 min. This treatment, which we have now used for 2 years, effectively prevents inactivation of dilute angiotensin II solutions during the course of a day's experiment.

Even more hazardous and less easy to detect is the activation of angiotensin I which can occur while the solution is being used. We have also seen activation in the tubing of infusion pumps so that, even though the reservoir contains angiotensin I, a much more active solution is delivered after passing through the pump. This activation, which may represent conversion by bacteria, is also prevented by the procedure I have outlined.

Without such antibacterial precautions, there is the strong possibility that, during an acute experiment, solutions of angiotensin II will become less active and those of angiotensin I will become more active. It is hardly necessary to stress how such changes will affect results in which conversion of angiotensin I to II is being measured by a comparison of their potencies.

Another serious hazard in the use of synthetic peptides is the presence of biologically active or inactive impurities synthesized during manufacture and not thereafter excluded. One manufacturer quotes ">95% chemical purity" for the angiotensins and for bradykinin and its analogues. Analysis of a sample of one of these peptides, methionyl-lysyl bradykinin (A. C. Camargo, J. R. Pinto, and L. J. Greene, manuscript in preparation) shows the presence of 20% lysyl bradykinin and about 3% bradykinin. Such contamination with other biologically active substances, often with widely differing potencies, must substantially affect interpretation of comparative results. Even a 5% impurity can be important. For example, the ratio of potencies of angiotensin I and II on the rat colon preparation is about 1 : 16. A 5% contamination of the angiotensin I sample with angiotensin II will double its apparent activity.

To eliminate these problems I suggest that biologists working with peptides such as the angiotensins and bradykinins use the following procedure. (1) Prepare, store and use solutions of the peptides in conditions which minimize the possibility of bacterial contamination. We have found the antibacterial procedure described above to be adequate. The use of bacitracin has been suggested by Pickens *et al.*², but addition of preservatives to solutions may introduce further biological activity³. (2) Check the solutions by bioassay both at the beginning and end of an experiment, until they are sure their precautions are adequate. (3) Perform and publish experiments to check the biological purity of the synthetic peptide. A comparison of the potencies of the angiotensins in a particular tissue or organ is more reliable when their purity is validated by effects (a) on blood pressure after intravenous injection, to show the relative activity of angiotensin I after conversion to angiotensin II, and (b) on an isolated organ such as the rat uterus or rat colon, as an indication of lack of conversion of angiotensin I before use. (4) Insist that the manufacturers of the peptides define clearly and more exactly the constitution and amounts of possible contaminants in their products. These figures, together with the batch numbers, should also be published.

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Carcinogenic Effect of a Dimethyl Sulphoxide Extract of Betel Nut on the Mucosa of the Hamster Buccal Pouch

THE high incidence of oral cancer in the Far East is generally attributed to the habitual use of betel nut "quids" which may contain, as well as betel nut, cured tobacco and various other organic and inorganic materials¹⁻⁴. Attempts to extract from betel nut a material carcinogenic for oral tissues have not been successful^{5,6}, possibly because extracts were prepared with solvents in which the carcinogenic materials were not soluble. We have therefore extracted betel nut, alone or in combination with cured tobacco, with dimethyl sulphoxide (DMSO) and applied the extracts in this vehicle. Our choice of DMSO was based on the evidence that it is an excellent solvent with a low toxicity⁷, that it has great powers of penetration⁸ and that it enhances the absorption of various drugs through skin and mucous membranes without apparently affecting their pharmacological properties⁹⁻¹². Furthermore, the use of DMSO as a vehicle decreases the latency period for the production of oral tumours in hamsters with dimethylbenzanthracene¹³⁻¹⁴. We have found that repeated, topical applications of DMSO extracts of betel nut to the mucosa of the buccal pouch of hamsters result in the development of leukoplakia and tumours. The incidence of tumours increases when the extract is made from a mixture of betel nut and cured tobacco, although extracts of tobacco alone cause leukoplakia but not tumours.

Extracts were prepared as follows. Betel nut (Banarsi Pan Shop, Jammu, India) was hand-ground in a porcelain mortar, and 30 g of the ground material mixed with 10 ml. of DMSO (Aldrich Chemical Co., Cedar Knolls, New Jersey) in a glass flask and kept in the cold (4°-5° C) for 48 h. The contents of the flask were then transferred to a porcelain mortar, hand-ground to a paste and thoroughly mixed with 10 ml. of DMSO. The mixture was stored at 4°-5° C in a glass flask for 24 h and strained through cheesecloth and stored at 4°-5° C for future use in a glass-stoppered, amber bottle. Extracts of a mixture of betel nut and tobacco were prepared in a similar fashion with 5 g of cured tobacco (Banarsi Pan Shop), ground by hand and added to the ground betel nut before the first mixing with DMSO. Extracts of tobacco alone, with 5 g of hand-ground tobacco serving as the starting material, were similarly prepared and stored.

Table 1 Occurrence of Leukoplakia and Tumours in Hamster Buccal Pouches after Chronic Topical Application of Dimethyl Sulphoxide (DMSO) Extracts of Betel Nut and of a Betel Nut and Tobacco Mixture

Treatment	No. of hamsters with leukoplakia	No. of hamsters with tumours
DMSO (11)	0	0
Tobacco (12)	8	0
Betel nut (21)	19	8
Betel nut and tobacco (21)	18	16

The number of hamsters is given in parentheses.

Male, golden Syrian hamsters (Dennen Animal Industries, Gloucester, Massachusetts), approximately 9 weeks old, were housed in pairs in small mesh wire cages over a bedding of 'Pellicel'. Their diet consisted of laboratory chow and water given *ad libitum*.

Extracts and control solution (DMSO) were applied to the right buccal pouch mucosa with separate camel hair brushes three times a week for 21 weeks. Before each application, the bottles of extracts and control solution were allowed to reach room temperature. In the course of each application, the mucosa of the buccal pouch was examined for gross pathological alterations.

Table 1 shows that the application of extracts of betel nut alone resulted in the development of tumours in 38%, and of

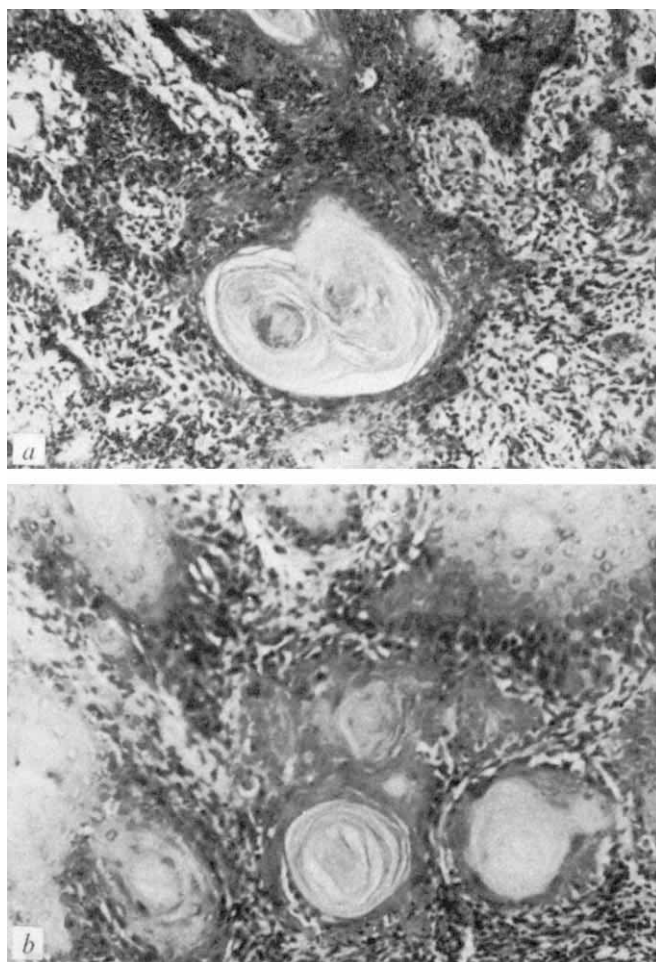


Fig. 1 Microscopic appearance of buccal pouch mucosa after chronic application of the DMSO extracts. *a*, Betel nut extract stained with haematoxylin and eosin ($\times 15$). Epithelial cells show loss of polarity. There is dyskeratosis with "pearl" formation. This tumour was classified as a squamous cell carcinoma, grade II. *b*, Betel nut and tobacco extract stained with haematoxylin and eosin ($\times 15$). Epithelial pearl formation is very marked. Cell polarity is lost. There is marked enlargement of cells and of their nuclei. Under higher power, numerous mitotic figures were clearly evident. This tumour was classified as a squamous cell carcinoma, grade III.

leukoplakia in 90%, of the hamsters receiving the extract. Application of the extract prepared from the mixture of betel nut and tobacco resulted in the development of tumours in 76% of the group. Extracts made from tobacco caused leukoplakia in 66% of the group, but no tumours were observed. Topical application of the control solution (100% DMSO) did not result in the development of leukoplakia or tumours. The cumulative incidence of leukoplakia and tumours during the 21 week experimental period is shown in Table 2. The data indicate an earlier appearance of tumours in the hamsters

Table 2 Time Course of the Appearance of Leukoplakia and Tumours during the Application of Extracts of Betel Nut and of the Betel Nut and Tobacco Mixture

Time (weeks)	Leukoplakia		Tumours	
	Betel nut (%)	Betel nut and tobacco (%)	Betel nut (%)	Betel nut and tobacco %
1-6	0	10	0	0
7-9	19	24	0	10
10-12	38	38	14	33
13-15	52	57	24	57
16-18	76	76	38	76
19-21	90	86	38	76

receiving the extract made from the betel nut and tobacco mixture. In both groups, all tumours had developed by the end of 18 weeks. The incidence of leukoplakia, on the other hand, was still increasing at the end of the experimental period.

By gross observation, leukoplakia developed in the form of patches that had a bluish-white or milky-white appearance. Early in their development leukoplakia lesions were not indurated but later they developed a clearly elevated margin and became rough to palpation. Leukoplakia appeared before tumours, and tumours developed only in hamsters with leukoplakia. Grossly, the tumours were of the papillary type and had a nodular appearance. They seemed to grow more rapidly in hamsters that received the extract made from the betel nut and tobacco mixture. The extent of growth of the tumours also appeared to be greater in this group, and some of the tumours reached a very large size and bled quite easily when the camel hair brush was applied.

At autopsy, the hamsters were killed with ether and right and left buccal pouches were dissected, fixed in 10% neutral formalin, sectioned in paraffin and stained with haematoxylin and eosin. Submandibular lymph nodes were also removed for histological study. Microscopic examination revealed that the tumours were squamous cell carcinomas with a variable degree of malignancy (Fig. 1). Examination of the lymph nodes revealed no signs of metastases.

DMSO can solubilize so many compounds that it is not possible to make a reasonable speculation about the identity of the active carcinogenic materials in betel nut. It seems likely that they are fairly potent, for they were effective in producing pathological lesions in spite of the simplicity of the method used to prepare the extracts.

It has been suggested that lime salts in the betel quid eaten in some countries are the carcinogen⁶; our results do not support this suggestion, for lime salts were not added to our extracts. Nor, according to our results, is the tobacco in the quid the source of the carcinogenic materials¹⁵. They suggest, however, that the tobacco contains materials which, although not themselves carcinogenic, can enhance the carcinogenic actions of substances present in betel nut. But it is possible that a carcinogenic action of the tobacco extract could have been demonstrated in a longer experiment or if a more potent extract had been utilized.

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Epidermal Antigens in Cutaneous Dysplasia and Neoplasia

THE loss of tissue-specific antigens, which has been considered to be important in neoplastic behaviour¹⁻³, occurs in both experimental and human tumours, including squamous cell carcinomas of human^{4,5} and murine⁶ origin. Techniques used to demonstrate antigen deletion have involved heterologous anti-tissue sera or naturally occurring organ-specific auto-antibodies. Among the latter, the discovery of anti-skin activity in the sera of human subjects with the skin diseases pemphigus vulgaris⁷ and bullous pemphigoid⁸ has provided reagents for the demonstration of antigenic change in experimental skin dysplasia and neoplasia.

We have assessed antigenic patterns in the epidermal elements by the indirect immunofluorescence method⁹ using (a) pemphigus vulgaris serum, which stains specifically the intercellular areas and cell membranes^{7,10} of stratified squamous epithelium, particularly of the stratum spinosum, and (b) bullous pemphigoid serum, which reacts specifically with the basement membrane zone⁸ of stratified squamous epithelium. Prevention of immunofluorescent staining by serum absorptions with human epidermis, but not other tissues, confirmed the specificity of the reactions studied.

Dysplastic and neoplastic lesions were induced in the skin of hairless mice by five applications of a 0.5% solution of 3-methylcholanthrene in benzene at 14 day intervals¹¹ or by daily irradiation for 1 min with ultraviolet light of wavelength 2800-3200 Å. The histology of the various skin lesions which developed was correlated with the pattern of epidermal antigens. Epidermal hyperplasia developed in 1-2 months, dysplastic changes in 2-5 months, and squamous cell carcinomas from 4 months onwards. Both ultraviolet light and 3-methylcholanthrene induced similar skin lesions, with the same antigenic patterns, although the changes occurred earlier with 3-methylcholanthrene.

In simple hyperplastic lesions, antigen deletion was not observed—the intercellular and cell membrane immunofluorescence was uniform throughout the entire thickness of the epidermis, which varied from four to eight cells, and the underlying basement membrane zone stained evenly throughout its length. In dysplasias, characterized by focal areas of three to six irregular layers of heaped up cells in the basal region of the epidermis, a loss of intercellular and cell membrane antigenicity was detected by immunofluorescence (Fig. 1). Conventional histological examination showed that the morphology of the antigen-negative areas was that of premalignancy. The cells were pleomorphic, had frequently lost their polarity and their squamous maturation was poor; a clear perinuclear halo zone was often seen in the cytoplasm and the nuclei were hyperchromatic. The fluorescent staining of the adjacent basement membrane zone, however, remained essentially normal.

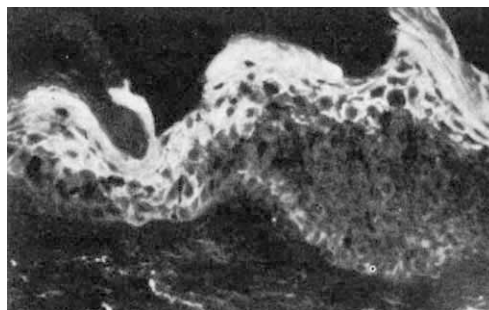


Fig. 1 Immunofluorescent staining by pemphigus serum of dysplastic hairless mouse epidermis. Staining is absent in dysplastic epidermis in contrast to bright fluorescence in adjacent areas. ($\times 200$.)

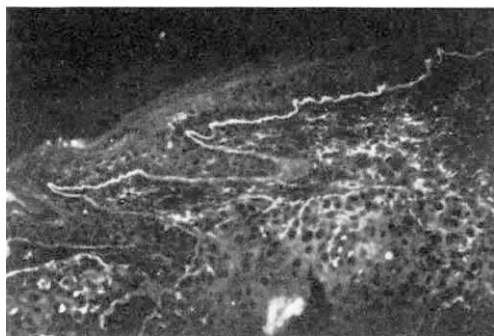


Fig. 2 Immunofluorescent staining by bullous pemphigoid serum of squamous cell carcinoma, which in part is deep to normal epidermis. Basement membrane zone of normal epidermis shows bright fluorescent staining, except where neoplastic epidermal cells project into dermis. Tumour mass exhibits only flecks of staining, chiefly non-specific. ($\times 120$.)

With the development of squamous cell carcinoma and invasion of the underlying dermis, the intercellular and cell membrane antigen in many areas of tumour was either diminished or lost. This change was most obvious in the deeper parts of the tumour, and the invading edge was always totally depleted of antigen. In some adjacent areas, however, tumour cells had differentiated, with normal intercellular and cell membrane staining. With tumour invasive growth, staining of the basement membrane zone became thinned, irregular, and in parts was absent (Fig. 2), particularly where the tumour was penetrating the deeper parts of the dermis.

We conclude that the loss of intercellular and cell membrane antigens in this experimental system is an index of delayed or impaired differentiation associated with premalignant and malignant change in proliferating epidermal cells. The antigen-negative cells demonstrate impaired differentiation as judged by conventional criteria and were those which had invaded the dermis. Some tumour cells retained the capacity to undergo squamous maturation and produced intercellular and cell membrane antigen, but these were never observed at the invading tumour margin. Loss of basement membrane zone antigen seemed to accompany invasion of the dermis by the antigen-negative tumour cells. Within the tumour, the basement membrane zone antigen was retained whenever adjacent cells exhibited squamous maturation together with the intercellular and cell membrane antigen.

Our results are consistent with the view that antigenic deletion is an important factor in the local invasion by malignant cells. This experimental system provides a useful model for studies of epidermal differentiation and dedifferentiation and the role of these processes in the maintenance or breakdown of tissue homeostasis.

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Homotransplantation of Isolated Epiphyseal and Articular Cartilage Chondrocytes into Joint Surfaces of Rabbits

THE limited capacity for repair of mature articular cartilage damaged by arthritis, trauma, infection or tumour has stimulated many clinical and experimental investigations into methods of replacing joint cartilage with different types of homotransplants.

Skeletal homografts were used by Lexer in 1925 in man using half joints and whole joints from amputation or fresh cadavers¹. Results of this type of grafting were poor because of the difficulty in fixation of the graft and partial or total rejection by the recipient². Volkov has reported³ the clinical use of massive half-joint osteoarticular grafts in 105 cases with survival of graft in 60%, but results with twenty whole joint homotransplants were poor as a result of inadequate fixation and rejection by the recipients. In animals, homotransplantation of articular cartilage with a thin layer of subchondral bone 2–3 mm thick has been successful in the hip and knee but, in spite of some encouraging reports, the technical and immunological problems make the widespread clinical use of such grafts impossible at present^{2–10}.

A different approach to the replacement of articular surfaces was made by Chesterman and Smith⁶ following the isolation of adult articular cartilage chondrocytes in suspension by Smith in 1965¹¹. It had been shown previously that there was no rejection of the cells of this type of graft when they were implanted into bone. This was thought to be caused by either the avascularity of the cartilage tissue or the possible protective function of the mucopolysaccharide in the matrix in preventing access of antibodies to the cells deep in the transplant^{5,12}. Isolated chondrocytes in suspension were placed in a V-shaped defect cut in the upper articular surface

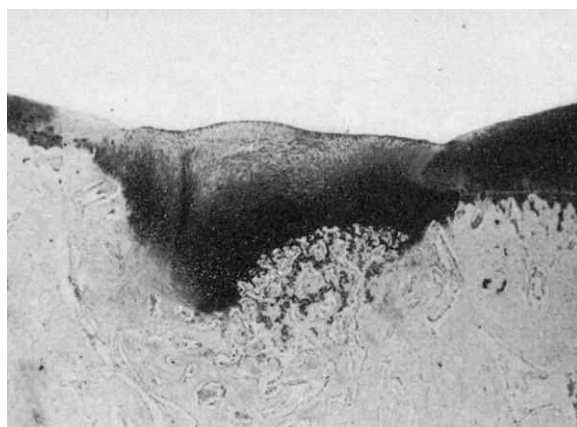


Fig. 1 Graft of epiphyseal cells into a drill hole in the upper articular surface of the rabbit tibia. Deep metachromatic staining of the matrix of the cells in the defect can be seen with endochondral ossification in the base of the graft after 4 weeks. (Toluidine blue, $\times 2.5$.)

of the rabbit humerus with a dental saw. Examination of the joints 6 weeks later revealed that although the transplanted cells survived, they were surrounded by fibrous tissue and filling of the defects did not occur. The authors of the work thought that the formation of fibrous tissue around the grafts was probably caused by the exposure of subchondral bone marrow in the base of the defect. But in the course of the same study chondrocytes prepared in a similar manner and implanted into the cancellous bone of the iliac crest of host rabbits survived, produced matrix and became incorporated.

There may have been other reasons for failure of the grafts into joint surfaces. Chondrocytes in suspension are difficult to control physically and many may not have stayed in the defect. The dental saw used to cut the defect may have caused burning and death of the cartilage and subchondral bone and prejudiced survival of the transplanted cells. Moreover, isolated adult articular cartilage cells may not have the capability to survive and produce matrix in the mechanically unfavourable environment of a moving joint.

In view of these considerations we decided to proceed with further studies using isolated chondrocytes as homotransplants. Laurence and Smith¹³ had demonstrated that isolated epiphyseal and articular chondrocytes survived when implanted into fractures in rabbits. It seemed to us that epiphyseal chondrocytes and articular cartilage cells from young animals would have more potential for repairing defects in cartilage surfaces in view of their known capacity for division which ceases when animals reach skeletal maturity^{14,15}.

Epiphyseal cells were obtained from the metatarsal epiphyseal plates of young male New Zealand White rabbits

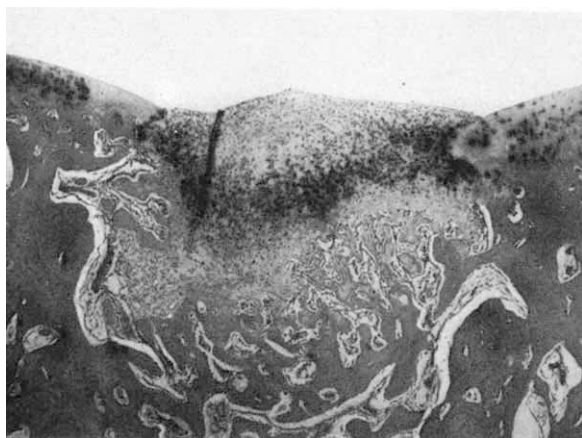


Fig. 2 35S autoradiograph of same specimen as in Fig. 1 shows labelling of the matrix in the same distribution as the toluidine blue stain verifying the viability of the grafted cells. ($\times 2.5$.)

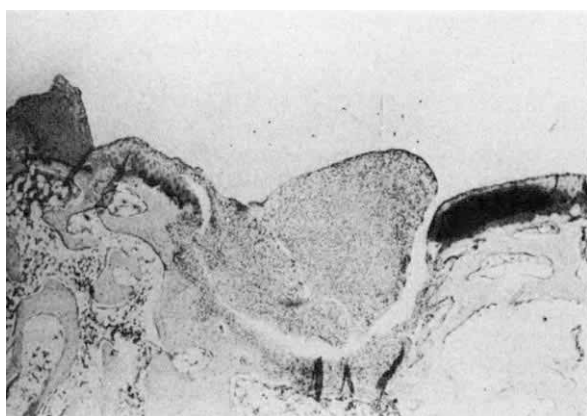


Fig. 3 The control drill hole at 4 weeks is filled with a plug of fibrous tissue which does not show metachromatic staining. (Toluidine blue, $\times 2.5$.)



Fig. 4 Graft of epiphyseal cells into upper articular surface of the tibia 8 weeks after implantation. The cells fill the defect, are producing matrix and seem to be incorporated into the defect. Endochondral ossification is occurring at the base of the graft and there is no sign of inflammatory response. (Toluidine blue, $\times 2.5$.)

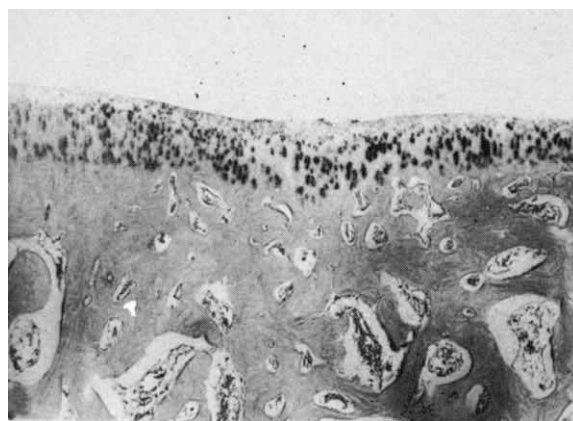


Fig. 5 35S autoradiograph of same specimen as in Fig. 4 shows uniform labelling of the grafted cells and the surrounding original articular cartilage.

weighing between 250 and 350 g. The periosteum was removed and the epiphyses were cut into thin slices with a scalpel. The slices were placed in a flask containing Eagle's basal medium and 0.25% trypsin and were stirred with a magnetic stirrer for 20 min. Separation of the cells from the matrix was completed by treatment of the cartilage for 30 min in Eagle's basal medium containing 0.025% collagenase stirred similarly. The cells were spun down and samples were checked for viability by the trypan blue technique.

Graft recipients were adult male New Zealand White rabbits weighing between 2.5 and 3.5 kg. Their knee joints were exposed under general anaesthetic and a hole 3 mm wide and 3 mm deep was drilled in the lateral tibial articular surface using a hand drill. The cells in suspension were pipetted into the defect. Nine animals received epiphyseal cell grafts in this way. The joints were closed and the animals were then left free in their cages. The opposite knee was used as a control; the drill hole was made in exactly the same manner but no cells were instilled into the defect.

In a parallel experiment articular cartilage was removed in slivers with a scalpel from the lower femoral articular surface of the knees of young rabbits weighing 250–350 g. The cartilage was cut into pieces approximately 2 mm across and the cells were separated from the matrix by successive exposure to 0.25% trypsin and 0.25% collagenase as in the first experiment. The isolated cells were instilled into drill holes in the lateral tibial articular surface of nine adult rabbits. The

opposite knee was used as a control, the hole being drilled in the same manner but no cells were instilled into the defect.

The animals were killed in two groups 4 weeks and 8 weeks after grafting. The upper ends of the tibiae were excised and incubated for 3 h at 37° C in Eagle's basal medium containing 50 μ Ci ml.⁻¹ of sulphur³⁵. The specimens were washed, fixed in neutral formalin and decalcified in ethylenediamine-tetraacetic acid (EDTA). Sections (6 μ m thick) were cut through the grafted area and stained with haematoxylin and eosin and with alcoholic toluidine blue to show the presence and distribution of acid mucopolysaccharides. Autoradiographs were prepared by the dipping technique using Kodak NTB₃ emulsion and were kept in light-proof boxes at 4° C for 2 weeks. After development, autoradiographs were stained with haematoxylin and eosin.

After 4 weeks the defects in the tibiae which had received a graft of epiphyseal cells were still almost filled with translucent material. Histologically each drill hole was obvious but was filled to the level of the original cartilage. The cells occupying the base of a defect were cartilage cells arranged randomly in lacunae and the surrounding matrix stained deeply metachromatically with toluidine blue indicating that the cells were producing a matrix containing acid mucopolysaccharide (Fig. 1). At the base of the defect it seemed that endochondral ossification was occurring in the cartilage mass. Around the margin of the drill hole the matrix of the grafted cells was blending with the original cartilage and there was no sign of inflammatory reaction suggesting rejection in the subchondral bone. The autoradiographs showed labelling of

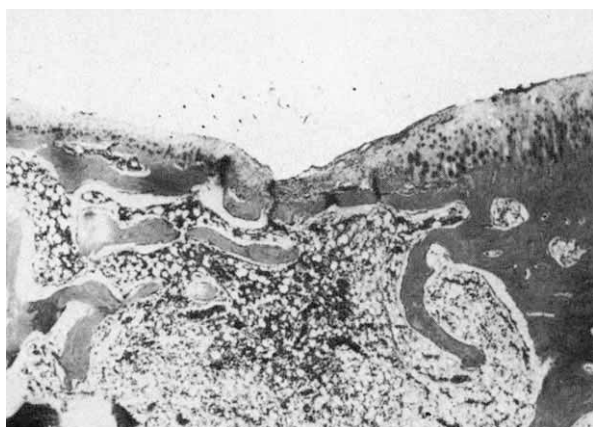


Fig. 6 The control drill hole shows incomplete filling with fibrous tissue after 8 weeks. (Autoradiograph, $\times 2.5$.)



Fig. 7 Graft of articular cartilage cells into a drill hole in the upper articular surface of the tibia. Cartilage cells are seen in the centre of the defect with a faintly metachromatic matrix but there are inflammatory cells and fibrous tissue around them after 4 weeks. (Toluidine blue, $\times 2.5$.)

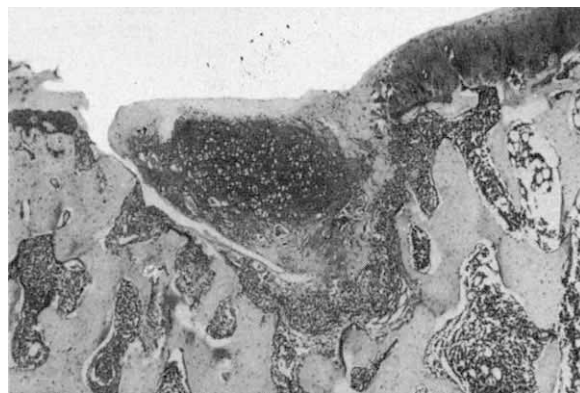


Fig. 8 Graft of articular cartilage cells into a drill hole in the articular surface of the tibia after 8 weeks. A clump of cells, with deeply staining matrix, are seen but inflammatory cells and fibrous tissue surround it and it is not becoming incorporated. (Toluidine blue, $\times 2.5$.)

the cells within the drill hole with ³⁵S in the same distribution as the metachromatic staining (Fig. 2). These cells were taking up ³⁵S and incorporating it in the matrix as part of the chondroitin sulphate molecule. The control sections showed a plug of fibrous tissue in the defect which neither stained metachromatically with toluidine blue nor took up the ³⁵S label (Fig. 3).

Eight weeks after epiphyseal cell grafting, drill holes were filled with material indistinguishable macroscopically from the surrounding cartilage. Histologically there was complete filling of the defect with chondrocytes and metachromatic matrix, which were becoming organized into columns in the deeper part similar to those in the surrounding cartilage. Endochondral ossification was occurring in the base up to the level of the original subchondral bone. The autoradiographs confirmed that the grafted chondrocytes were taking up ³⁵S and the labelling of the cells was comparable with that of the surrounding cartilage (Figs. 4 and 5). The control defect showed incomplete filling with fibrous tissue (Fig. 6).

In the articular cartilage group, after 4 weeks, there were cartilage cells in lacunae in the depths of the defect but the metachromasia of the matrix stained with toluidine blue was faint. There were inflammatory cells and fibrous tissue formed around the cells also (Fig. 7). After 8 weeks, cartilage cells were present in a clump in the defect and showed heavy staining of the matrix with toluidine blue, but they were not incorporated into the defect, and inflammatory cells and fibrous tissue were present around them suggesting that rejection was occurring (Fig. 8). This appearance was similar to that described by Chesterman and Smith⁶.

These experiments suggest that homografts of epiphyseal cells in suspension can survive, produce matrix and become incorporated into defects made in the tibial articular surface of adult rabbit knees. Moreover, the cells in the defects became arranged after 8 weeks into the three layers characteristic of articular cartilage and endochondral ossification occurred in the base of the graft. By contrast the articular cartilage cell homotransplants, although some cells apparently survived, became surrounded by inflammatory cells and fibrous tissue and did not become incorporated into the defects.

It is probable that the cells in the defect are of donor origin, but they may arise from the inductive effect of epiphyseal cells on the medullary cells of the host causing the recipient cells to differentiate into chondrocytes. Bridges¹⁶ showed that the hypertrophic cells of the growth plate are inductive to osteogenesis. This dilemma might be answered by studying the effect of using epiphyseal cells devitalized by slow freezing and thawing or by labelling the donor cells with tritiated thymidine before transplantation.

Whatever the mechanism by which the defects are filled, it seems that isolated epiphyseal cells are superior to articular cartilage cells as homotransplants in rabbits. It may be that this type of graft will prove useful clinically in the treatment of arthritic joints, particularly if a method of long-term storage of epiphyseal cells can be devised.

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Use of Hydrated Polyphenylene Oxide Sulphonate Ion Exchange Membranes for Artificial Kidneys

ION exchange membranes of hydrated polyphenylene oxide sulphonate 0.2 mil thick have been used successfully to purify brackish water and other aqueous salt solutions by reverse osmosis¹. Water permeabilities ranged from 0.02 to 0.5 gal-mil/foot²-day-atm., with salt rejections of dissolved salts in the range of 70 to 95%. Water permeability was directly proportional to the water content of the membrane. Preliminary investigations with highly polar organic solute molecules which ionize little in water suggest that permeability of these species also increases with increasing water content of the membrane. As the water content of the membrane decreases, interaction of the solute molecule with the membrane functional group becomes important.

For a high water content PPO-SO₃H membrane, rejection of organic molecules increases with increasing molecular weight of the species. This can be seen from the reverse osmosis results in Table 1. The excellent rejection of very high molecular weight solutes such as albumin, combined with the decreased rejection for low and intermediate molecular weight organic species, suggests that PPO-SO₃H is an excellent candidate for a haemodialyser membrane.

We have investigated the use of hydrated polyphenylene oxide sulphonate (PPO-SO₃H) membranes in artificial kidneys by measuring their resistance to mass transfer of urea and creatinine. We also studied a perfluorocarbon sulphonate ion exchange membrane ('R' membrane), a Bemberg 'Cuprophane PT-150' membrane and an Avisco 'wet gel' ultrathin

membrane². The last is regenerated cellulose which has not yet been dried in the process of cellophane manufacture. The percentage water content of the PPO-sulphonate membrane was 62; those of the other three were 23, 55 and 75 respectively. All measurements were made with ¹⁴C labelled tracer solutes in aqueous solution at 37° C, using a batch dialyser operating at 100 or 200 r.p.m. Details of the experimental procedure have been described before^{2,3}.

Table 1 Rejection of Organic Molecules by Reverse Osmosis

Organic constituent	Feed conc. (mg/l.)	Molecular weight	% organic rejected
Urea	100	60	20
Lactic acid	75	90	42
Creatinine	100	113	54
Sucrose	1,000	342	85
Human serum albumin	100	67,000	> 99

Table 2 Membrane Permeability Data

Membrane	Wet thickness (mils)	True resistance to mass transfer (min/cm)	D _{eff} (cm ² /s × 10 ⁶)
Urea			
JE-128-S-A (PPO-SO ₃ H)	0.25	1.9 ± 1.1	5.7 ± 3.3
ABL-1 ('R' membrane)	0.50	21.1 ± 1.5	1.0 ± 0.1
'Cuprophane' (PT-150)	1.10	16.3 ± 1.0	2.86 ± 0.23
Avisco 'wet gel' (Ultrathin)	2.59	18.2 ± 1.0	6.02 ± 0.45
Creatinine			
JE-128-S-A (PPO-SO ₃ H)	0.25	5.2 ± 1.5	2.1 ± 0.6
ABL-1 ('R' membrane)	0.50	34.8 ± 2.0	0.61 ± 0.05
'Cuprophane' (PT-150)	1.10	30.3 ± 1.4	0.843 ± 0.068
Avisco 'wet gel' (Ultrathin)	2.59	30.6 ± 1.4	3.58 ± 0.24

Figures show measured value ± s.d. All runs were made at 37° C. Effective diffusion in the membrane is defined by

$$J = \frac{\Delta C}{R_m} = D_{\text{eff}} \frac{\Delta C}{t_{\text{wet}}}$$

Diffusivity of solute in water at 37° C: urea, 1.80×10^{-5} cm²/s; creatinine, 1.14×10^{-5} cm²/s.

A summary of the membrane permeability data is given in Table 2. As a result of its higher water content, the wet gel is significantly more permeable on an equivalent thickness basis than 'Cuprophane'. It is weak, however, and unavailable in thickness less than 2.6 mils (wet). The 'R' membrane has a higher mass transfer resistance than 'Cuprophane' on an equivalent thickness basis. The PPO-SO₃H membrane has a much lower mass transfer resistance than 'Cuprophane'. On an equivalent thickness basis, it is comparable with the wet gel membrane but it is 1/10 as thick.

Recently, several highly efficient haemodialysers have been developed with narrow, well defined blood flow channels and low blood mass transfer resistances. Analysis of clinical dialysance data for these devices gives overall mass transfer resistances as low as about 30 min/cm and 50 min/cm for urea and creatinine⁴. Since the blood, membrane and dialysate resistances are additive, replacement of 'Cuprophane' by a 0.25 mil PPO-SO₃H membrane would reduce the respective overall resistances to about 15 min/cm and 30 min/cm. The improvement in performance would permit considerable reduction in the size of these devices.

The PPO-SO₃H membrane is easily cast as a 0.1 to 1.0 mil unsupported film with excellent chemical and physical properties. Water contents can be adjusted easily to give the desired permeability characteristics by controlling the ion exchange capacity (mequiv. of sulphonic acid groups/g of dry polymer) of the sulphonated polymer. Larger values give membranes with larger water contents, all other variables being fixed. Techniques have also been developed for adjusting the water content of a sulphonated PPO membrane with a given ion exchange capacity. Further work should be conducted to optimize PPO-SO₃H for use as a haemodialyser membrane by defining the thickness, water content and ion exchange capacity needed to give membranes with good physical strength and which exhibit minimal mass transfer resistance to urea, creatinine and higher molecular weight organic species.

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Origin of Apparent Polyploid Spermatocytes in the Mouse

APPARENT polyploid primary spermatocytes at diakinesis-metaphase are seen frequently in air-dried preparations from testes of mice and other mammalian species¹. Most seem to be tetraploids but some higher "polyploids" occur, including "hexaploids" and even "decaploids". Deviation from the geometric series suggests that these cells, or at least some of them, may arise as a result of cell fusion rather than such processes as endoreduplication or the formation of restitution nuclei, which would double the chromosomes. Although apparent polyploid spermatogonia are also seen, such cells will not necessarily give rise to spermatocytes which can reach diakinesis-metaphase. Indeed, the possibility that polyploid germ cells, spermatogonia and spermatocytes are all an artefact of preparation arising from the spreading together of the contents of adjoining cells is not excluded on present evidence. Artefactual origin is nevertheless unlikely because of the uniformity in degree of contraction and appearance of the chromosomes or bivalents and the regularity of many of the spreads.

Apparent polyploid spermatocytes in structural homozygotes have not been observed to contain the multivalent associations which might have been expected had four or more homologues been present at zygotene pairing. But pairing, once initiated, could be completed so rapidly that primary independent pairing events rarely, if ever, occur at more than one site in one pair of homologues. If so, bivalents would result and multivalents should be absent, whatever the number of homologues at zygotene.

Animals heterozygous for a reciprocal translocation provide a potential test, for a minimum of three primary pairing events

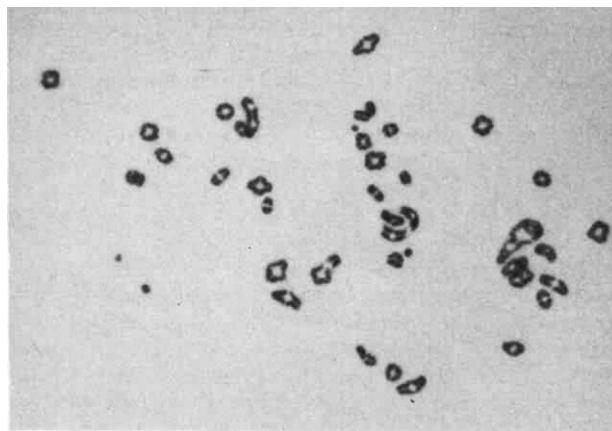


Fig. 1 Tetraploid oocyte from a T6 female heterozygote at first metaphase. There are five univalents, twenty-eight bivalents, one trivalent and four quadrivalents. Four of the multivalents involve homologues and one, shown at greater magnification in Fig. 2, is mixed. ($\times 1,080$.)

is necessary for the formation of a quadrivalent. If tetraploid spermatocytes exist before zygotene in a translocation heterozygote, the four-fold representation of each segment of the translocation complex would provide an opportunity for the formation of exceptional types of multivalent configurations, irrespective of the speed at which pairing is completed in specific pairs of homologous segments. Configurations of increasing complexity up to chain and ring octavalents would be anticipated.

Mouse translocation T26H is a radiation-induced reciprocal translocation between one of the longest and one of the shortest chromosomes (unpublished results of A. G. Searle, C. E. F. and C. V. Beechey). The breakpoints have been located genetically close to the agouti locus in linkage group V and about 11 cross-over units away from *Os* in linkage group XVIII on the side removed from sombre (personal communication from A. G. Searle). Cytological evidence that the segments exchanged were unequal is provided by the presence of a long marker chromosome in mitotic cells of heterozygotes (a second, short marker is also often recognizable), by the grossly different length of the arms of the pachytene cross configuration and by the inequality in length of the four components of the quadrivalents seen at diakinesis-metaphase. The latter are usually rings, but chain quadrivalents also occur frequently. Replacement of the potential quadrivalent by a trivalent plus univalent or by two bivalents is exceptional.

Table 1 Quadrivalent Configurations observed in Diploid and "Polyploid" Spermatocytes of Two Male T26H Heterozygotes at Diakinesis-metaphase

Mouse No.	Spermatocyte type	Configuration RIV	CIV *	% RIV
1	Diploid	158	42	79
	Polyploid	15	5	75
2	Diploid	206	194	52
	Polyploid	24	15	62

* Includes three diploid cells in which the quadrivalent was replaced by a trivalent and univalent and three in which it was replaced by two bivalents.

Observations of air-dried preparations² from two T26H male heterozygotes are summarized in Table 1. Ten apparent tetraploid spermatocytes were found and analysed in mouse 1, seventeen apparent tetraploids and an apparent decaploid in mouse 2. Thirty-nine ring quadrivalents and twenty chain quadrivalents, indistinguishable from those found in diploid cells, were recorded in these "polyploid" cells. There were no

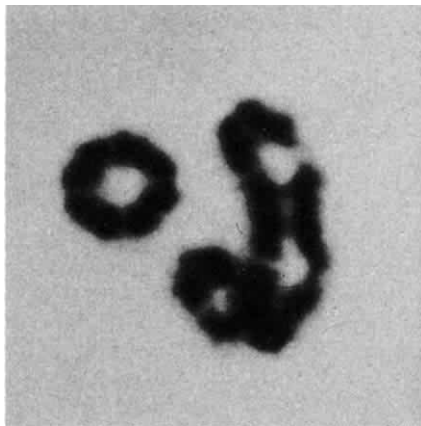


Fig. 2 Exceptional quadrivalent and neighbouring bivalent from Fig. 1, enlarged. ($\times 5,200$.)

exceptional configurations or multivalents of higher order. Moreover, the ratios of ring to chain quadrivalents do not differ significantly from the ratios in diploid cells (mouse 1, $\chi^2_1 = 0.17$, 0.9, $P < 0.8$; mouse 2, $\chi^2_1 = 1.58$, 0.3, $P < 0.2$), although the difference between mice is very highly significant ($\chi^2_1 = 41.44$, $P < 0.001$). The conditions of prophase pairing and chiasma formation must have been very similar in diploid and "polyploid" cells within the same mouse but different between mice.

Prophase conditions must have been different again in the one tetraploid oocyte we have seen in meiosis (Fig. 1). This was obtained from a female heterozygous for the T6 translocation and was recognized as a presumptive tetraploid cell from the size of the germinal vesicle before the preparation was made. Though most of the chromosomes are associated as bivalents, a few multivalents are present including an exceptional symmetrical quadrivalent derived from the doubled translocation complex (Fig. 2). This could be designated segmentally as *AB-BC-CB-BA*.

In spite of the presumptive difference in the conditions of meiosis between the sexes, it is difficult to account for an exceptional configuration formed in a tetraploid oocyte but not in tetraploid spermatocytes if primary meiotic pairing depends solely on attraction between specific homologous loci. And if it is postulated that there may be an additional general attraction (such that, for example, a segment *A* in one chromosome *AB* would have a greater likelihood of primary pairing with the homologous segment in the second *AB* chromosome rather than one in an *AD* chromosome) and that the attraction is stronger in the spermatocyte than the oocyte, it would be expected, in the limit, to lead to the formation of four completely homologous bivalents from the eight chromosomes of the doubled translocation complex, and yet not exclude the formation of exceptional multivalents. Our observations provide no support for this possibility, and conclude that the quadrivalent configurations seen in our T26H preparations were formed in diploid cells which then either fused together naturally or spread together during preparation to give the apparent polyploid cells observed.

This conclusion does not, of course, exclude the possibility that a few truly polyploid spermatocytes may be found in other conditions or in other species. The situation could be clarified by a micro-spectrophotometric investigation of DNA content of spermatocyte nuclei at different phases of meiosis and also by a search for polyploid cells at zygotene and pachytene. Tetraploid zygotene spermatocytes should be recognizable by their size and could be confirmed, in the mouse, by counts of the characteristic pairs of chromocentres. These are believed to represent the paired centric and (partly) paired terminal regions of the newly formed autosomal bivalents. About twenty pairs are present in normal diploid spermatocytes

at zygotene. We cannot recall having seen presumptive tetraploid cells in zygotene, or in pachytene, but have not made a special search for them.

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Physiological Transport in Relation to Ageing

It has been shown that the diffusivity of oxygen, glucose and carbon dioxide through blood plasma decreases with increased plasma protein concentrations^{1,2}. Navari *et al.*² and Connor *et al.*³ have further demonstrated that the diffusivity of oxygen and glucose is markedly affected by the concentrations of these proteins in human plasma within their normal physiological ranges. For example, an increase of the concentration of albumin from 2.0 to 5.0 gm/100 ml. at 37° C causes almost a 50% decrease in the diffusivity of oxygen. Since the concentrations of albumin and the globulins, as well as cholesterol, change with various pathological conditions and with ageing of "normal" subjects, subsequent changes in diffusion may have physiological consequences.

We have investigated the resulting change in diffusivity with increasing age. Information about the change in plasma protein with age was obtained from previous studies of healthy human subjects. Scatter diagrams were drawn to show mean concentrations of albumin, γ -globulin, fibrinogen and cholesterol against age. Plots were made of those which showed definite trends, and were then fitted with least-squares polynomials by the method of Hall and Canfield⁴. These results were used to predict the changes in diffusivity in plasma with age.

No trend was statistically apparent in either the albumin or fibrinogen plots; the arithmetic averages of the reported mean values were calculated to be 4.10 ± 0.32 g/100 ml. for albumin and 0.318 ± 0.099 g/100 ml. for fibrinogen. Data for albumin were obtained from various sources⁵⁻¹², and so were data for fibrinogen^{6,10,12-15}.

Although we found no statistical evidence of a decrease in albumin concentration with increasing age, Nöcker and Bemm²⁴ and Meindok²⁵ reported hypoalbuminaemia in elderly subjects. Further, the total protein content of plasma remained approximately constant with age (about 7.0 g/100 ml.) in spite of Soergel's finding that this concentration decreased with age in his subjects²⁶.

Although there is much literature about blood constituent concentrations in both healthy and diseased people, some of it refers to plasma and the rest to serum. The standard deviation of data for all constituents, however, is so large that there is little difference between citing a mean concentration in g/100 ml. of plasma or in g/100 ml. of serum (plasma has less than 0.5% fibrinogen; serum has none). We therefore made no distinction between data from plasma and data from serum.

Some data were reported in relative concentration units, that is, percentage of total protein. Where the amount of total protein was given, we converted it to absolute units. Otherwise we assumed a total protein content of 7.0 g/100 ml.

An increase in concentration with age was evident for both cholesterol and γ -globulin. The best fit polynomials for both of these were linear equations of the form

$$C_c = x_1 + Ay_1 \quad (1)$$

and

$$C_{\gamma-G} = x_2 + Ay_2 \quad (2)$$

where C_c is the concentration of cholesterol in g/100 ml.; $C_{\gamma-G}$ is the concentration of γ -globulin in g/100 ml.; A is age in years; x_1 is 0.1703; y_1 is 1.3846×10^{-3} ; x_2 is 0.8359, and y_2 is 3.5180×10^{-3} .

These equations are shown graphically in Figs. 1 and 2. Cholesterol data were obtained from previous reports¹⁶⁻²², as were γ -globulin data^{6,7,9,10-12}. The empirical relation found by Navari²³ for the prediction of diffusivity in human

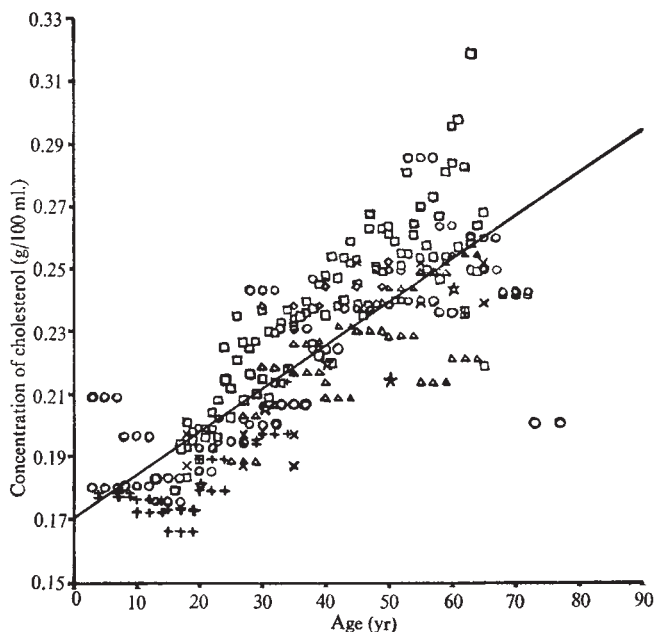


Fig. 1 Plasma cholesterol plotted against the age of "healthy" humans. Data are from the references as follows: $\circ$, 16; $\square$, 17; $\triangle$, 18; $\times$, 19; $+$, 20; $\diamond$, 21; $\star$, 22.

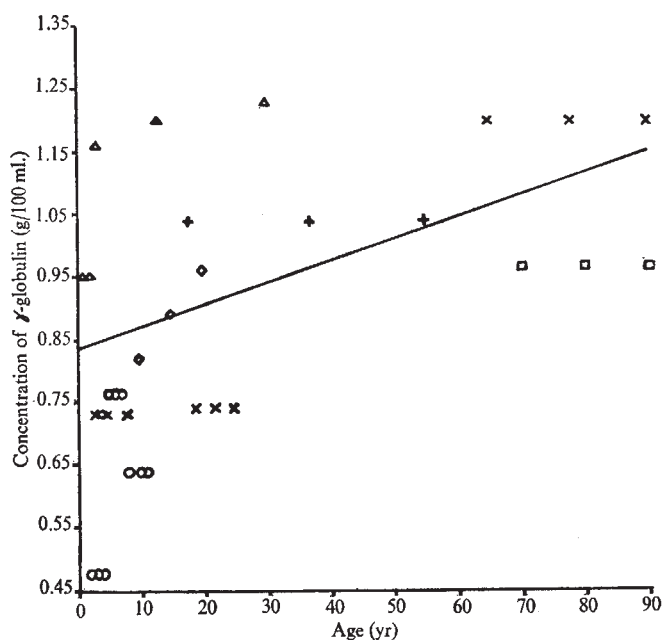


Fig. 2 γ -Globulin concentration in human plasma plotted against age. Data are from the references as follows: $\circ$, 12; $\square$, 6; $\triangle$, 11; $\times$, 7; $+$, 9; $\diamond$, 10.

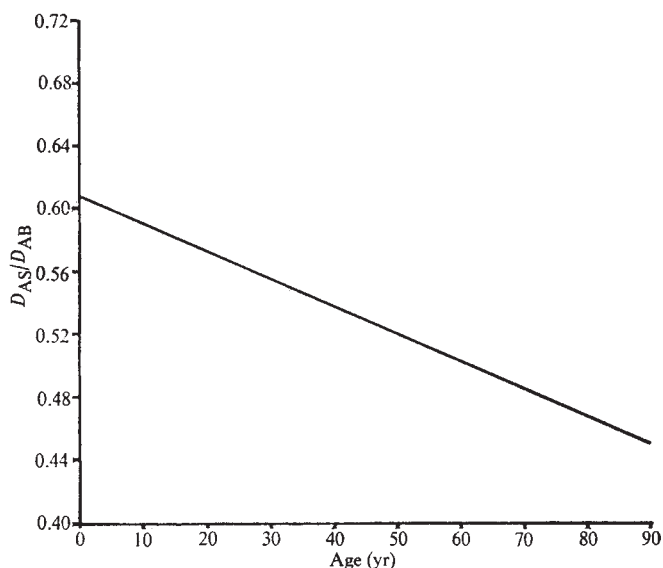


Fig. 3 Diffusivity (D) ratio of oxygen (A) through human plasma (S) and oxygen (A) through water (B) plotted against age.

plasma in the physiological concentration ranges is

$$D_{x-p}/D_{x-w} = (-0.178)(C_A - 2.0) + (-0.405)(C_{\gamma-G} - 0.70) + (-0.500)(C_F - 0.15) + (-0.251)(C_c - 0.10) + 1.14 \quad (3)$$

Here D_{x-p}/D_{x-w} is the ratio of the diffusivity of x (for example oxygen) in plasma to that in water. C_F is the concentration of fibrinogen in g/100 ml. and C_A is the concentration of albumin.

Substituting values for C_F and C_A and equations (1) and (2) into equation (3) yields the diffusivity ratio D_{x-p}/D_{x-w} as a function of age. The result (Fig. 3) is a steady decline in diffusivity with increasing age.

The physiological consequences of a decrease in the plasma diffusivity with age are perhaps more significant in the light of Sinha's work²⁷ demonstrating that a plasma layer 3 μ m thick offers at least as much resistance to the transfer of oxygen as the red cell membrane. For example, hypoxia resulting from decreased diffusivity of oxygen due to increased age might contribute to the physiological changes usually associated with ageing, such as loss of memory.

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Initial Crystallin Synthesis and Lens Fibre Formation are Independent of Lens Morphogenesis

If the part of a chick embryo destined to form the eye, the lens, part of the brain and the surrounding head region (Fig. 1) is removed before the lens has begun to develop and placed in liquid culture medium for 4 days, a structure resembling the lens will form appropriately near the presumptive retina^{1,2}.

Similarly the cells of the lens will synthesize the proteins unique to and characteristic of the lens (Figs. 2, 3).

Naturally, the lens forms in the following way. The head ectoderm adjacent to the optic vesicle (presumptive retina) adheres closely to it and then thickens. The optic vesicle is invaginated and so afterwards is the thickened lens placode, forming a hollow sphere which pinches off, free of the connecting ectoderm. Subsequently, the cells of the posterior (internal) portion of the sphere elongate to fill the cavity of the sphere. The result is a solid sphere with a thin epithelium anteriorly and elongated lens fibre cells posteriorly.

The lens of the cultured explant develops quite differently, but the final result is much the same. Instead of invaginating, the cells of the thickened ectoderm elongate directly, forming the characteristic lens fibres, correctly oriented (Fig. 4). The shape of the lentoid formed by this aberrant morphogenesis is similar to that of the normal lens in that the curvature of the anterior surface is greater than that of the posterior one and the dorso-ventral axis is about one and a half times longer than the dimension along the visual axis. We have measured neither change in volume of the lens cells with time, nor the total number of lens cells, so we cannot be sure whether fibre differentiation is restricted to cells in the original placode, whether proliferation of placode cells occurs or whether adjacent ectoderm cells migrate into the differentiating lentoid. Mitotic figures are frequently seen in the region of contiguity between the lentoid and the adjacent ectoderm, suggesting that cells are continually recruited into lens differentiation. In the final stages of lentoid differentiation, the adjacent attached ectoderm cells migrate out across the anterior surface of the lentoid, piling up in just that position they would have occupied if invagination had occurred.

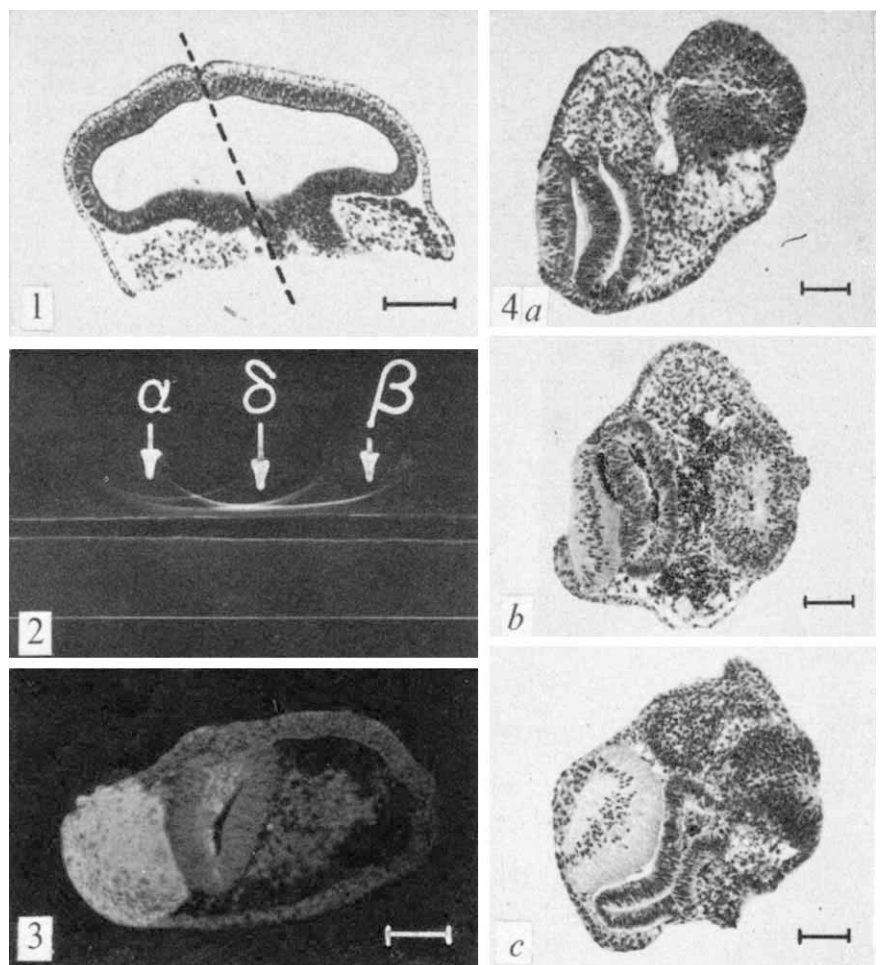
Of forty-one explants examined after 96 h in culture, thirty-two (78%) followed this aberrant morphogenetic pathway and

Fig. 1 Forebrain region of a 36 h chick embryo. This piece is cut along the line of neural fold fusion and explanted to liquid culture medium. The bar is 100 μ m long.

Fig. 2 Immuno-electrophoresis pattern of antiserum prepared against decapsulated adult chick lenses. Three major components are visible, alpha, beta, and delta (the first important soluble lens crystallin).

Fig. 3 Fluorescent antiserum indicates that lentoids forming in cultured explants synthesize at least one of the characteristic lens crystallins.

Fig. 4 Lens development in the explant at (a) 60, (b) 72, and (c) 96 h after removal to culture. The bar is 100 μ m long.



nine (22%) developed normally. No earlier investigators describing the differentiation of lens *in vitro* have called attention to this phenomenon¹, although Langman² figured, without comment, an explant forming a lens in culture which could only have arisen through abnormal morphogenesis.

Philpott and Coulombre⁴ reported that epithelial cells from 6 day chick embryo lenses would differentiate to form lens fibres in culture, which indicates considerable freedom from immediate environmental influence. Our experiment was done at an earlier stage when the lens was first forming, and indicates that ectoderm cells with no visible commitment to becoming lens cells undergo divergent differentiation nonetheless. McDevitt and Yamada³ have confirmed that fibre cell elongation by epithelium in culture is accompanied by the acquisition of the crystallin gamma which is characteristic of normal fibre cells of the amphibian.

Coulombre and Coulombre⁵ have shown that when lenses of chick embryos of 5 days incubation are reversed, so that the epithelium faces the retina, the former epithelium differentiates to form fibre cells. Although this suggests that lens morphogenesis is strongly influenced by the optic environment, our observations show that fibre cell formation does not depend on precedent lens vesicle morphogenesis, and corroborate the finding⁴ that proximity to the cornea does not inhibit fibre cell elongation.

It seems that elongation of ectoderm cells to form lens fibre cells is independent of invagination of the lens placode to form a hollow sphere, as is the synthesis of the first of the characteristic and unique lens proteins FISC (first important soluble lens crystallin). One might assume the antecedent event, spherulation, to be causally requisite and the elongation of the posterior cells of the sphere consequent on their position in the sphere and in relation to the optic vesicle. This seems not to be the case. It seems clear from this example that at least some of the biochemical and morphogenetic activities of embryos are rigidly and separately programmed long before their expression and are independent of immediately precedent events.

There is a correspondence in form between the aberrant lentoid and the normal lens developing in the egg. Although recent developments have clarified many of the apparently goal directed biochemical events in organisms^{6,7}, little is known about the regulation of form, and the broad tolerance of the embryo for perturbation. This extreme example of the acquisition of the appropriate form by an altogether different route emphasizes our ignorance of the terms in which developmental directives are specified.

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Fluorescent Glucoside in the Human Lens

A PROTEIN-FREE extract of the lens of man and other primates contains a fluorescent compound which is absent from the lens of the usual domestic and laboratory animal. This compound, detected on paper chromatograms by its reaction with ninhydrin, was believed to be a peptide¹. The protein free extract of the lens of man and certain other primates has been found to contain one or more fluorescent yellow pigments², and I have now isolated from the human lens the O-β-D-glucoside of L-3-hydroxykynurenine (Fig. 1), which is yellow and highly fluorescent. This glucoside has also been found in the silk-worm, *Bombyx mori*³.

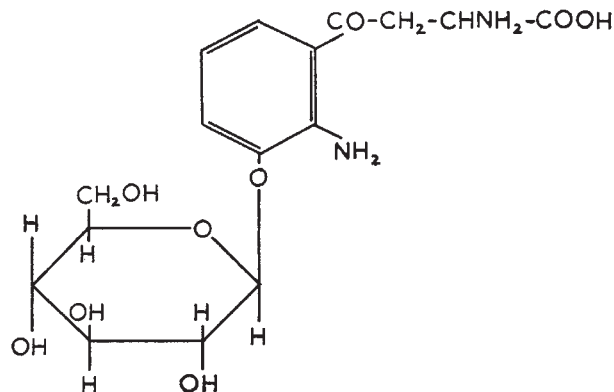


Fig. 1 Presumed structure of O-β-D-glucoside of L-3-hydroxykynurenine.

A protein-free dialysate of a dispersion of sixty lenses (about 8 g) obtained from cataract operations was concentrated *in vacuo* to a few millilitres and applied to a column of 'Sephadex G25' (140 × 1 cm). The column was eluted with 0.5% formic acid at a rate of about 0.2 ml./min. The fluorescent material was retarded on the column and could thus be largely separated from the bulk of amino-acids and so on. The fluorescent material which eluted from the column after about 90% of the ninhydrin-positive compounds had appeared was collected and concentrated to dryness *in vacuo*. The yellow deposit was dissolved in a little water and the highly fluorescent solution was streaked onto paper and subjected to electrophoresis at pH 1.6. One main and several subsidiary fluorescent bands separated, all positively charged at pH 1.6.

The main band (Fig. 2, F₂) was eluted with water and concentrated *in vacuo*. By treatment with β-glucosidase (emulsin: BDH Chemicals Ltd., Poole, Dorset), it was hydrolysed to give equimolar amounts of L-3-hydroxykynurenine and D-glucose (Fig. 2). The glucoside has a blue-white fluorescence on paper, whereas the 3-hydroxykynurenine has a weaker yellow fluorescence. This accords with the finding that the blue fluorescence of 3-hydroxykynurenine esterified in the hydroxyl position changes to a feeble yellow fluorescence when the 3-hydroxyl group is uncovered⁴.

The L-3-hydroxykynurenine was identified by comparison with the authentic compound (prepared from *Aku Aku* silk-worm larvae: Calbiochem), and with data in the literature, after electrophoresis at several pHs, chromatography in several solvents, and by means of several sprays and dips⁵⁻⁷. Its ultraviolet spectra in acid, neutral and alkaline solution were identical to those of the authentic compound, and also to published spectra. The action of kynureninase prepared from rat liver⁸ yielded alanine.

The glucose moiety was identified by chromatographic and electrophoretic techniques, and assayed enzymatically using hexokinase and glucose 6-phosphate dehydrogenase⁹, and also glucose oxidase (Boehringer).

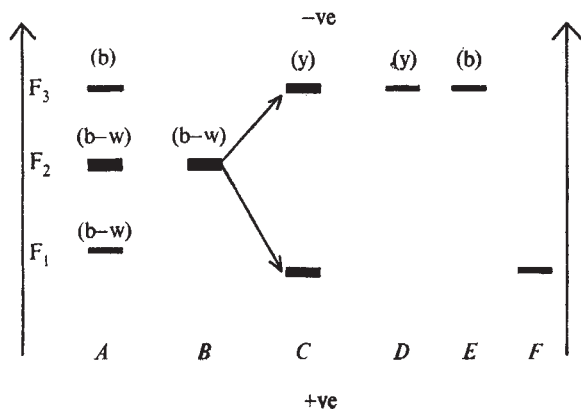


Fig. 2 Separation of the fluorescent compounds in the human lens by paper electrophoresis. Paper electrophoretogram pH 1.6 (formic acid : acetic acid : water; 125 : 375 : 2,000), 2.5 h, 300 V, No. 52 paper. A, F₁, F₂ and F₃ fluorescent compounds from lens; B and C, F₂ before and after treatment with β -glucosidase; D, E, and F, markers, 3-hydroxykynurenine, kynurenine and glucose. All compounds were located by fluorescence under an ultraviolet lamp (maximum emission 360 nm) except glucose (silver nitrate-NaOH)⁵. The colour of the fluorescence on paper is given: b-w, blue-white; y, yellow; b, blue.

The yield of the fluorescent glucoside corresponded to a concentration of about 0.65 $\mu\text{mol/g}$ of fresh lens. It was also found in normal (post-mortem) lens.

The two other fluorescent bands (Fig. 2, F₁ and F₃) were also examined. F₁ was also an O- β -D-glucoside, and the fluorescent moiety of the molecule was examined in the same way as 3-hydroxykynurenine. It was similar to the N-acetyl derivative of 3-hydroxykynurenine, except that it was not hydrolysed to 3-hydroxykynurenine by treatment with dilute acid or by 6 N HCl at 108° C for 16 h. It was not a substrate of kynureninase. The α -amino-group seems to be blocked (not by acetylation but in some other way).

F₃, the fastest moving compound, was shown to be kynurenine and this was confirmed by the fact that the action of kynureninase yielded anthranilic acid and alanine.

If the glucosides are formed within the lens, which seems probable, the reaction is an exception to the generalization that glucosides are not formed in mammalian tissues¹⁰.

It is reasonable to speculate that pathological changes in senile cataract in man could lead either to the failure of glucoside formation, or to the release of hydrolytic enzymes which split the inactive conjugate. These changes would result in the appearance of an active "tanning" agent 3-hydroxykynurenine, such as occurs in the formation of the insect cuticle¹¹ or of ommochromes in the insect eye¹², the oxidation product(s) of which could be involved in the formation of the brown fluorescent water-insoluble material in the nucleus of a black cataract¹³ (cataracta brunescens).

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Effect of a Bright Light Flash on Dark Adaptation of Human Rods

A BRIEF flash of bright light bleaches half the rhodopsin in the rods of a human eye, although Rushton found it as effective in elevating the rod threshold as a long full bleach¹. This is a paradox because in both rat² and man³⁻⁶ there is a linear relation between the fraction of visual pigment still unregenerated and the log threshold in the dark after a full bleach. Fig. 1 illustrates this effect. The open circles show the dark adaptation curve after a long full bleach, the filled circles following a 1/1,500 s xenon flash which yielded 1.5 (10)⁸ hv absorbed/rod and bleached half the rhodopsin exposed to it.

The curves in Fig. 1 have the familiar two branches showing the recovery first of cones, then rods. I am here only concerned with rods. The differences between filled and open circles in Fig. 1 within the period that rods can be studied are no greater than those found between two repetitions of the same experiment. The filled circles agree more exactly with the open circles than they do with the triangles, although the latter is the curve obtained after a long exposure to a dimmer light which bleached the same amount of rhodopsin as the xenon flash.

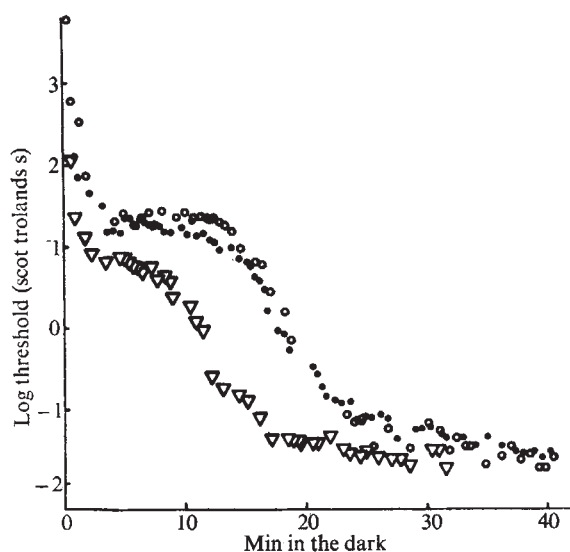


Fig. 1 Dark adaptation curve for the 6° temporal retina of the normal eye; after (i) a long (190 s) full (6.1 log scot trolands) tungsten bleach (open circles); (ii) a short xenon flash (filled circles) and (iii) a long (190 s) partial (4.55 log scot trolands) bleach (triangles). The rhodopsin bleaches produced by the xenon flash (Honeywell Strobosonar 65A) and the weaker (4.55 log scot trolands) long exposure were the same, but the flash bleach is followed by a rod dark adaptation curve identical to that which follows the long full bleach. This is Rushton's paradox. The test was a 1° blue square (dominant wave length 453 nm).

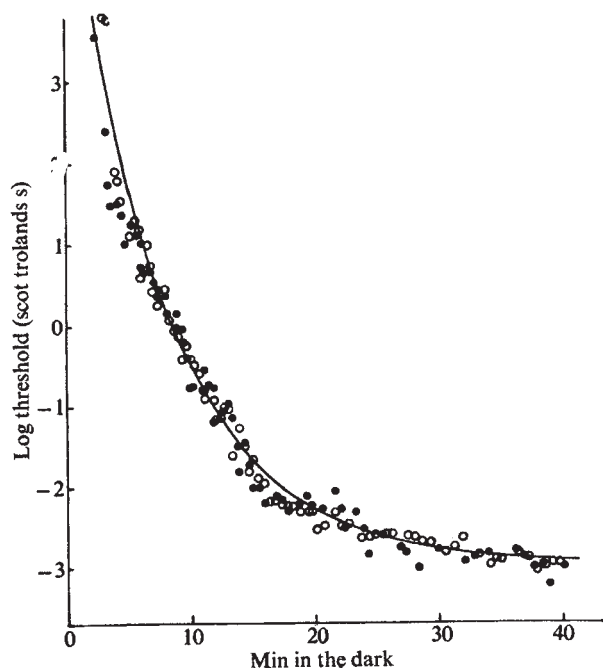


Fig. 2 Rushton's paradox in the rod monochromat. The filled circles represent the recovery after a long full bleach, the open circles after the xenon flash. The test was a blue (dominant wave length 453 nm) 2.5° square. The smooth curve is a single exponential curve.

The current explanation of this paradox is due to Dowling and Hubbard⁷. Using the albino rat retina, which consists chiefly of rods, they could measure rod thresholds almost immediately after bleaching. Changes in log threshold were proportional to changes in rhodopsin concentration throughout dark adaptation after both bleaches. Initially, the threshold after flash bleaching was 1.5 $\log_{10}$ units lower (and the rhodopsin concentration was 35% higher) than after a long full bleach. The recovery curve of rhodopsin (and log threshold) after the latter was exponential (time constant, 50 min), but that after a flash was S shaped. After 30 min in the dark, the S and exponential curves joined and went together during the next 2.5 h, as the final 50% of the pigment regenerated and sensitivity increased 1,000-fold.

In man the earliest phases of rod recovery are obscured by cones and so, according to Dowling and Hubbard⁷, the flat part of the S-shaped rod dark adaptation curve is hidden. Unfortunately, the matter is not so simple: for human cones the early part of the dark adaptation curve is uncovered, but neither the log threshold nor pigment regeneration follows an S curve after flash bleaching^{8,9}.

Rods are not cones and the normal human retina with four visual pigments is more complicated than the rat retina¹⁰. I have therefore taken the matter up again by studying a rod monochromat, an observer with normal rods but very few cones¹¹. (These cones—like those of the albino rat¹²—have the action spectrum of rhodopsin¹³.)

The recovery of the rod sensitivity after a long full bleach can be followed for a wider than normal range when an area of the monochromat's retina which has few cones is tested. The filled circles in Fig. 2 show one example of this. The curve has the same form as the recovery of sensitivity after the xenon flash (open circles), though the flash bleached half and the long exposure bleached all of rhodopsin in the test area. As closely as the threshold can be measured, the two curves in Fig. 2 go together.

The results in Fig. 2 are typical of ten similar experiments. None show S-shaped log threshold recovery curves after flash bleaching. With my apparatus it was not possible to make measurements larger than 7.0 log units above the absolute rod

threshold, so that for the first few minutes (the exact interval varies from one experiment to the next) in the dark, the test light could be made bright enough to obtain a measurement.

In view of the way rhodopsin regenerates after the two kinds of bleaches (Fig. 3), it is unlikely that 1.5–3 min during which I could not measure it, the threshold recovery after flash bleaching deviated from that which follows a long full bleach, as Dowling and Hubbard predicted.

Fig. 3 shows that rhodopsin regenerates after full bleaches along an exponential course with a time constant of nearly 400 s (similar to the rod dark adaptation curve). It recovers much more quickly, however, after flash bleaches: rod monochromats and normals behave identically in this respect. Unlike Dowling and Hubbard's results with the rat, the curves showing resynthesis after the two bleaches (which produce identical rod dark adaptation curves) never overlap. In this respect, human rods resemble human cones^{8,9}.

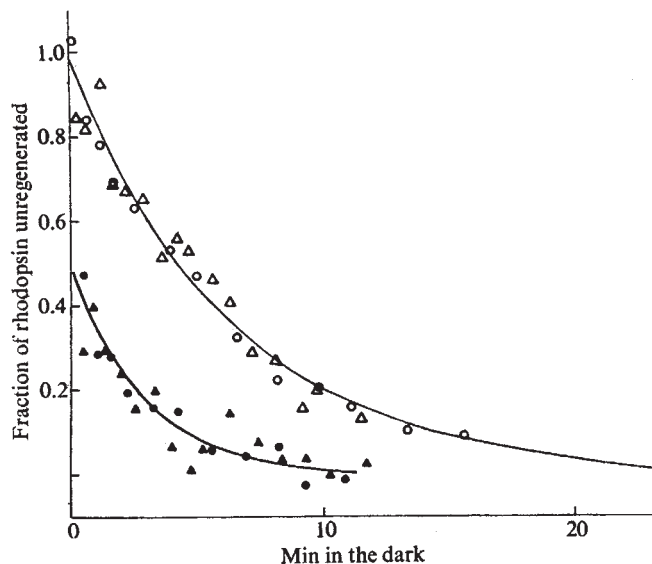


Fig. 3 Regeneration of rhodopsin as measured by retinal densitometry after the two bleaches which gave the dark adaptation curves illustrated by the circles in Figs. 1 and 2. The filled symbols show resynthesis after xenon flash bleaches; the open symbols resynthesis after 190 s 6.1 log scot trolands. The circles are for the normal, the triangles for the rod monochromat. The smooth curves are exponentials; that one through the filled symbols has a time constant of 165 s, that through the open symbols, 380 s. Measurements were made with a slightly modified version of the Florida retinal densitometer of Rushton and Hood¹⁶. The modifications are described elsewhere¹⁷. The measuring light was 555 nm.

My results show that the explanation of Dowling and Hubbard does not resolve Rushton's paradox in man, and raise doubts about the validity of the albino rat retina as an experimental model for human rod adaptation. For example, it now seems possible that the human retina—unlike that of the albino rat—has enzymes which isomerize one of the labile intermediaries back to rhodopsin while the all *trans* chromophore is still attached to the chromophoric site. If this were the case, it could account for the fact that resynthesis of rhodopsin *in vivo* is about one order of magnitude faster in man than in rat.

But I do not know how, in man, two such different bleaches produce identical rod dark adaptation curves. There is evidence^{14,15} to suggest that the simple relation between rhodopsin resynthesis and the decrease in log threshold in the dark after a full bleach has a complex physiology, but none of it is so direct as that presented here.

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Excretion and Retention of Stable Strontium in Children

THE turnover of stable strontium, a minor mineral constituent of the diet, chemically similar to calcium, has been studied in man in order to compare the metabolic behaviour of strontium and calcium and to estimate the accumulation of fall-out strontium-90 in the body, ingested at a constant rate over a long period. So far, little information has been obtained from studies of children because the experiments have involved giving doses of the radioactive isotope. Measurements of the residence time of strontium in children aged less than one year have been obtained, however, by comparing the amounts of natural strontium ingested and excreted^{1,2}, but for the study of older children this method is unreliable because the accumulated strontium approaches a state of equilibrium attained in adult life. In previous work in this laboratory, the accumulation of strontium and calcium in four children aged 4-14 yr was studied by giving doses of 100 mg of natural strontium³, but because the normal dietary intake at these ages is only 1 mg a day, these large doses may have disturbed the balance of strontium in the body and invalidated the conclusions of the experiments. In the work reported here, we have overcome this problem—and at the same time avoided giving doses of radioactive isotopes to children—by using stable strontium enriched in ⁸⁴Sr (Oak Ridge Laboratories). The information on strontium turnover could thus

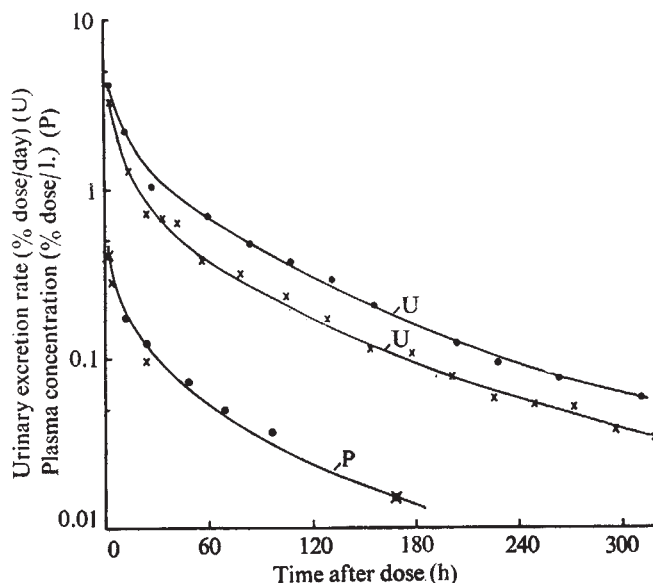


Fig. 1

be obtained whilst the subjects consumed a normal diet with a normal content of natural strontium.

An oral dose of 5 mg of Sr enriched in ⁸⁴Sr was given at 11.00 h to two boys aged 10.2 and 12.3 yr who were medical in-patients of the St Mary's Teaching Group of Hospitals. Urine and faeces were collected daily for 14 days and samples of venous blood were taken at 2, 4 and 24 h and 6 and 7 days after the dose of ⁸⁴Sr enriched strontium. All samples were dried to ash and a known weight of the ash was irradiated with thermal neutrons in one of the Harwell reactors simultaneously with known weights of natural Sr and enriched ⁸⁴Sr. The marker Sr as well as the natural Sr content of the samples was estimated in the manner described before⁴.

The change in the rate of urinary excretion of the ⁸⁴Sr marker with time and the corresponding changes in the plasma concentration of the marker in the boy (L. D.) and in an adult are shown in Fig. 1. The total urinary and faecal outputs of the marker are shown in Table 1 together with the derived value for the percentage of the marker retained. For comparison, the mean retention of an oral dose of natural Sr for three children³ and three adults⁵ is also given. These results confirm the conclusion reached earlier³ that the total excretion of Sr by children aged 4-14 yr and adults given a single dose is not significantly different, although the relative urinary and faecal excretion of the Sr differed markedly in the two age groups. There is also a similarity between the results obtained from the two experiments on children although the oral dose of strontium in one was twenty times that in the other. It may be concluded that the excretion and retention of Sr are controlled largely by the intake and excretion of Ca, which were very similar for the two groups of children.

The plasma concentrations and the rates of excretion in the urine of natural Sr and Ca were measured as well as those for the marker Sr given in Fig. 1. Table 2 gives the plasma concentrations and the rates of urinary excretion with the respective renal clearances. This table includes ten children

Table 1 Excretion and Retention of Strontium after a Single Oral Dose

Subject	Oral dose of Sr (mg)	Age (yr)	Period of study (days)	Excretion urinary	% dose faecal	Retention % dose
L. D.	5	10 3/12	14	4.0	73.3	22.7
M. L.	5	12 4/12	14	1.7	82.0	16.3
Three children	100	4 10/12-14 4/12	16	3.0	77.7	19.3
Three adults	100-250	29-54	16	18.5	64.8	16.7

Table 2 Plasma Concentration and Contemporary Urinary Excretion Rate for Strontium and Calcium with the Respective Renal Clearance in Children and Adults

Subject	Age (yr) and sex	Diagnosis	Strontium		Calcium		Renal clearance		Relative renal clearance CSr/CCa
			Plasma ($\mu\text{g per l.}$)	Urinary excretion ($\mu\text{g/day}$)	Plasma (mg per l.)	Urinary excretion (mg/day)	CSr l./day	CCa	
G. B.	4 M	Small stature	28.2	21	108	14	0.74	0.13	5.7
P. C.	5.6 F	Periodic syndrome	35.3	92	104	69	2.6	0.66	3.9
R. C.	5.6 M	Encopresis	43.5	97	98	56	2.2	0.57	3.9
L. S.	8.3 F	Abdominal pain	41.0	73	108	52	1.8	0.48	3.7
S. D.	8.5 M	Obesity	28.5	102	102	113	3.6	1.1	3.3
J. W.	9.0 M	Small stature	39.0	39	97	17	1.0	0.18	5.6
P. O'D	9.0 F	Abdominal pain	31.0	139	105	132	4.5	1.26	3.6
S. B.	9.5 F	Recurrent headache	32.6	94	98	87	2.9	0.89	3.3
E. W.	9.9 M	Arthralgia	26.0	177	101	207	6.8	2.05	3.3
L. D.	10.2 M	Obesity	19.0	139	96	310	7.3	3.2	2.3
M. L.	12.3 M	Bronchitis	40.7	201	104	198	5.0	1.9	2.6
A. C.	13.8 M	Sickle cell anaemia	28.5	261	96	305	9.2	3.2	2.9
—	—	Mean	32.6	120	101	130	4.0	1.3	3.7
—	—	Range	19–44	21–261	96–108	14–305	0.7–9.2	0.13–3.2	2.3–5.7
8 adults	35–63	Mean	29	290	97	344	9.7	3.2	3.0
		Range	16–43	144–535	89–100	128–640	7.4–12.7	1.8–5.8	2.2–4.0

who were the subjects of a previous study but for whom the data now presented were not included in the original report⁴. For comparison the corresponding means of results obtained from experiments with eight adults are also included in the table⁶.

For children the renal clearance for Sr and Ca is significantly less than for adults, the urinary excretion rates of these elements being much lower and there is evidence of an increase in these values with age. The significant results are perhaps that the relative renal clearance $C_{\text{Sr}}/C_{\text{Ca}}$ or renal discrimination between Sr and Ca in children is about the same as the corresponding value in adults, and that the plasma concentration of Sr, which like Ca is approximately constant, is only slightly higher than in adults.

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Phosphatized Ostracods from the Cretaceous of Brazil

SOME well preserved ostracods were first observed by Dr C. Patterson, British Museum (Natural History), in the insoluble residue of a carbonate nodule during the acetic acid preparation of the enclosed fish skeleton. Subsequently, careful examina-

tion of further washed residues produced more than 180 specimens all belonging to the same ostracod species. Nearly all the complete carapaces contained appendages and, in the males, copulatory organs projecting below the carapace in a partially erect position (Fig. 1A).

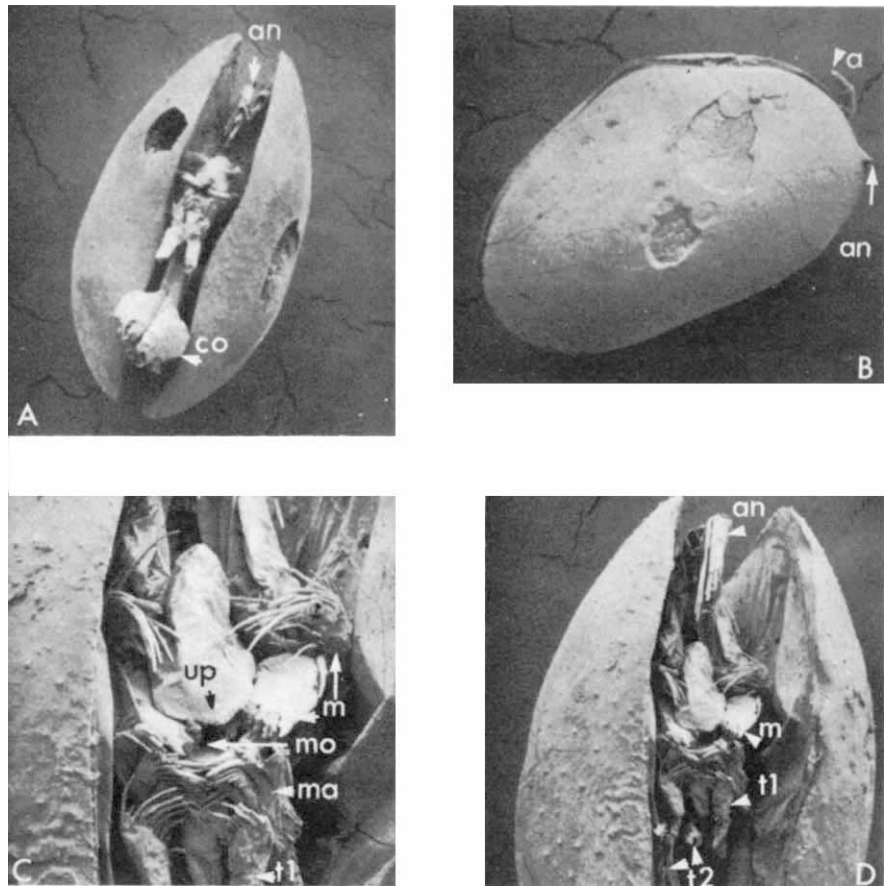
The nodule was obtained from the Santana Formation, a marl and gypsiferous succession developed in the Serra do Araripe, Ceará, northern Brazil. The formation, originally considered to be of Turonian age¹, is now placed in the Aptian/Albian^{2,3} and represents a freshwater deposit. No marine ostracods were found in the nodule and the species obtained (so far unnamed) belongs to the freshwater subfamily Cypridinae, family Cyprididae. Other nodules examined were crowded with the same ostracod but these, being calcareous, dissolved in the acid. Although a calcite infilling was often present inside the ostracod carapace, the shell, appendages and body of the animal were clearly resistant; the state of preservation suggesting a mineral replacement of the original chitin. An X-ray powder analysis, undertaken by Dr R. Davies (BM(NH)), revealed that both the shell and the appendages were preserved as apatite.

Previous records of fossil ostracod appendages^{4–6} have been disappointing because they have lacked morphological detail. The appendages of the Brazilian ostracod are so well preserved because the original chitin has been replaced by apatite. This mineralization has retained the detail missing from the purely chitinous fossil remains recorded so far.

The finding of appendages in fossil ostracods is important because complete information on the ostracod makes possible a more accurate assessment of its phylogenetic position in relation to living species. It is important to know whether morphological similarities in the carapace between fossil and living ostracods may be accompanied by similarity of appendage detail.

In living representatives of the Cyprididae the first thoracic appendages are modified for feeding⁷, often being referred to as a second pair of maxillae. Examination of the fossil first thoracic appendages (Fig. 1C) shows that the adaptation for use as auxiliary feeding appendages rather than as walking limbs was in existence approximately 100 million years ago. The length of the antennules (Fig. 1B) suggests that the ostracod was able to swim, at least for short distances, and the powerful antennae used for crawling and in the search for food indicate that it also had a bottom dwelling existence. The upper lip has a serrated, hardened central portion and the teeth of the mandibles are well developed. Both the maxillae and the first thoracic appendages were used to push food—probably algae and decaying plant and animal tissue—into the mouth. The locomotor appendages (Fig. 1D) are long,

Fig. 1 *A*, Ventral view, male carapace, $\times 50$; *B*, right side, female carapace, $\times 50$; *C*, mouth and associated appendages, female carapace, $\times 140$; *D*, ventral view, female carapace, $\times 70$. *a*, Antennule; *an*, antenna; *m*, mandible; *ma*, maxilla; *mo*, mouth; *co*, copulatory organ; *t1*, first thoracic appendage; *t2*, second thoracic appendage; *up*, upper lip.



terminating in a claw with (where preserved) a long spine. In addition to the male copulatory organ internal casts of the testes have been observed. Many specimens also have intact the muscles attaching the body to the inside of the shell.

From the examination of the complete carapaces, the ostracods seem to have died fairly quickly as a result of asphyxiation. This is indicated by their limbs and other appendages being situated in the life position and the valves agape. It is suggested that the ostracods, closely associated with the skeleton of the fish *Cladocycus gardneri* Agassiz (on which they could have been feeding at the time), were suddenly buried and asphyxiated by the putrefying fish. The sediment in which they were buried would become locally enriched with phosphate salts and within a matter of days the chitin of the shell and body, together with the calcium carbonate of the shell, was replaced by apatite. The time factor for the replacement is controlled by the fact that the muscles, which would have decayed very rapidly, are also preserved. There is, in fact, no evidence of decay in these specimens at all. A more detailed examination of this unique ostracod is currently being undertaken.

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Is the New Composite Cranium from Swartkrans a Small Robust Australopithecine?

Clarke, Howell and Brain have recently presented evidence which suggests that more than one hominid taxon is represented at Swartkrans in South Africa¹. They demonstrated that parts of a cranium and most of the left portion of a face, SK 847, fit together with a left temporal SK 846b and the maxilla SK 80. The maxilla was originally placed in the taxon "*Telanthropus capensis*"², but the genus was subsequently sunk and the specimens were transferred to *Homo erectus*³, and later inappropriately suggested for membership in "*Homo habilis*"⁴.

There has been a negative reaction to the claim that the combined specimen SK 80/846/847 represents a more "advanced" taxon at Swartkrans^{5,6}. Both Robinson^{7,8} and Tobias⁹ had described SK 846 and SK 847 as a member of the robust australopithecine taxon "*Paranthropus*", and Robinson⁷ used it in comparisons of the "*Paranthropus*" range at Swartkrans with Olduvai hominid (OH) 5. SK 847 is one of the most complete undistorted faces from Swartkrans. Robinson has used a line drawing of it (ref. 8, Fig. 5b), along with one of SK 52 (ref. 8, Fig. 5a), to picture the Swartkrans maxillary variation overlap with OH 5. Tobias has indicated⁹ two facial characteristics which could distinguish faces of robust australopithecines from those of *Homo erectus*: (1) the anterior extent of the masseteric impression on the zygomatic arch and (2) the length and thickness of the supraorbital torus. In the first, an obvious component of the masticatory complex, the only australopithecine cranium more robust than SK 847 is OH 5. In the second, SK 847 has a far smaller supraorbital torus than any of the eight *Homo erectus* specimens for which the feature can be observed. The torus is at the small end of the australopithecine range which only overlaps at its large end with *Homo erectus*.

Do any features make SK 80/846/847 more like *Homo erectus* than the other specimens at Swartkrans generally considered

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robust australopithecines? What follows summarizes the relevant comparisons.

(1) Should we expect to find robust australopithecine crania at Swartkrans as small as SK 80/846/847? In Fig. 1, I compare symmetric reconstructions of SK 12, one of the largest mandibles from Swartkrans, and SK 74a, one of the smallest. Both mandibles have been designated "*Paranthropus*" by Broom and Robinson¹⁰. These reconstructions demonstrate that the size range at the site is greater than the size difference between male and female gorilla. SK 15, a "*Telanthropus*" mandible, is supposed to be small enough to fit the new cranium¹. SK 74a is smaller than SK 15 in all external dimensions, and is identical in alveolar arch length and breadth⁵. Disregarding the material described as "*Telanthropus*", therefore, the mandibular size range among the "*Paranthropus*" specimens at Swartkrans indicates that a cranium as small as, if not smaller than, SK 80/846/847 is to be expected. Fig. 2 shows a comparison of symmetric reconstructions of both SK 80/846/847 and SK 74a. The alveolar and mandibular dimensions indicate that the two specimens are identical in size.

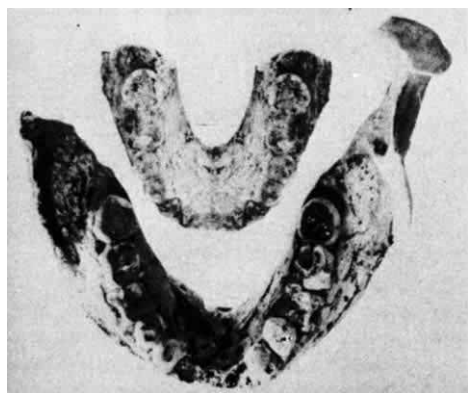


Fig. 1 SK 12, one of the largest mandibles from Swartkrans, compared with a symmetric reconstruction of SK 74a, one of the smallest. Both have been described as robust australopithecine. M_3 in SK 74a is missing.

(2) In general, *Homo erectus* differs from the australopithecines, both robust and gracile, in being larger but more gracile in appearance. Specifically, although almost all cranial dimensions of *Homo erectus* are greater than those of australopithecines, the facial structures associated with the masticatory apparatus are less robustly developed. If SK 80/846/847 is compared with the other crania from Swartkrans, however, the cranium is smallest in all dimensions but equally or more robust in masticatory associated features^{5,6} (see Table 1).

(3) The facial breadths of the composite cranium are less than all other robust australopithecines. Outer orbital breadth falls outside the *Homo erectus* range (102 mm–151 mm, $n=7$), as does the inner orbital breadth. Upper facial height is less than any other robust australopithecine—it is almost identical to that of the reconstructed Choukoutien female *Homo erectus* (based on skull 11). The composite cranium has a far more developed lower face and zygomatic process. The nasospinale-alveolar distance is 33 mm, compared with 26 mm for the reconstructed Choukoutien female. Outer orbital breadth is 124 mm for the *Homo erectus*, and midfacial breadth is about 95 mm. Breadth expansion of the face, and other diagnostic *Homo erectus* features, are not shown by SK 80/846/847. In comparison, the Chad hominid has facial breadths almost identical to those of the largest robust australopithecine, OH 5, but a facial height much less¹². This specimen makes a far more likely candidate for an early *Homo erectus*, given almost any possible reconstruction of the missing alveolar region.

(4) The principal evidence for extensive masticatory adapta-

tion in SK 80/846/847 lies in the massive zygomatic process⁵ and the very anterior masseteric attachment⁹. The ratio of the projective zygomatic bone height (Martin number 48(3a)) to the orbito-alveolar height (Martin number 48(3)) is only exceeded by OH 5 (Table 1). The robust Broken Hill Neanderthal has a ratio of 47.

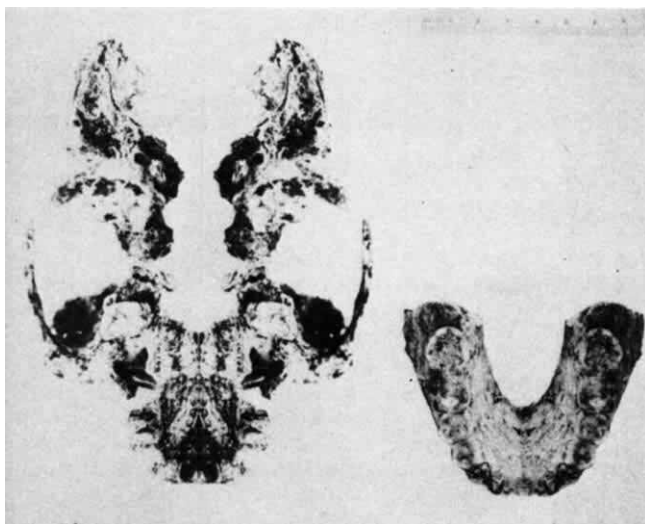


Fig. 2 Symmetric reconstructions of Swartkrans mandible SK 74a and composite cranium SK 80/846/847. The dimensions indicate a close size approximation of both the mandibular body and the dental arcade with SK 80/846/847. A wider reconstruction of the cranium makes it too big for the robust australopithecine mandible.

(5) The nasal bones follow the pattern of all robust australopithecines, except OH 5, being narrower at the superior margin (4 mm) and wider at the inferior margin (12 mm). The superior margin is very narrow for a robust specimen; four others range from 9 mm to 12.7 mm. The superior margin is well known in *Homo erectus*; five specimens range from 16 mm to 18 mm.

(6) The distances from porion projected in the median sagittal plane all indicate that the composite Swartkrans cranium is smaller than any *Homo erectus* specimen. The angle of the face seems to indicate prognathism for the specimen. I have attempted to calculate a very rough index of prognathism by dividing the porion-prosthion distance by the porion-nasion distance. This index shows SK 80/846/847 to be less prognathic than any other robust specimen.

(7) The facial and cranial dimensions of the composite Swartkrans cranium and the gracile australopithecine STS 5 are almost identical. The Sterkfontein specimen has a slightly broader cranium. The cranial length of SK 80/846/847 is unknown. Upper facial height, alveolar height, and all of the facial breadths except bijugal are within 1 mm of each other. The projected distances from porion to nasion and to glabella are identical. The distances to nasospinale and to prosthion, on the other hand, are significantly greater in the Sterkfontein specimen: 106 mm and 134 mm respectively. The relative lack of prognathism in SK 80/846/847 could possibly account for the projecting nasal bones of this specimen¹³. The zygomatics of the Swartkrans composite are both relatively and absolutely greater than those of STS 5, and the masseter attachments of the former are farther anterior and more robust. Similarly, the ratio of projective zygomatic height to orbito-alveolar height is only 48 in STS 5. Finally, the angle of the forehead with the supraorbital torus is greater in the Sterkfontein specimen.

(8) The supraglabellar portion of the SK 847 frontal is unfortunately missing, and the left lateral portion of the frontal fragment terminates after about 2 cm. Thus, it is not known

Table 1 Comparison of Cranial Measurements of Australopithecine and *H. erectus* Material

	SK 80/ 846/847	12	46	48	52	TM 1517	Koobi Fora 158	OH 5	Chad	OH 9	1	Java 2	Lantian	3	10	Choukoutien 11	12	13
Upper facial height	76		79	80	86		85	112	> 71									
Alveolar height	33	38	29	29	30	28	36	43	> 28									23
Orbito-alveolar height	48	64	54	62	55	52	65	75										
Alveolar/upper facial heights	43		37	36	35		42	38	39									
Outer orbital breadth	93		102	111	108		114	116	116	134	102	110	151	104	118		119	
Inner orbital breadth	83		88	94	90	84	98	100	102	116			143	95	98	90	106	
Bijugal breadth	98		97	119	124	118	142	123	117									
Midfacial breadth	88	113	97	95	94	106	115	122	93									
Sagittal projections:																		99
porion-prosthion	116	145	146	120	130	134	135	148										
porion-nasospinale	96	116	118	94	103	119	109	123										
porion-nasion	85		101	81	84	81	97	100		118		92		101	106	95	106	
porion-glabella	96		103	88	87		105	103		129		103		107	111	103	112	
p-Prosthion/ p-nasion	136		145	148	155	165	139	148										
Projective zygomatic/ orbito-alveolar height	60		48	48	54	50	42	62										
Bi-mandibular fossa breadth	90-95	145		130		120	150	140		140		120		129	144	128	125	
Supraorbital torus thickness	7.2		7.4	7.8	12.4		9.0	13.6	10.0	25.0	10.0	12.5	20.0	12.0	16.5	13.5	15.1	

All measurements are in mm. They were obtained from available casts, published figures and scaled photographs^{1,2,5,7,9-12}.

whether the specimen had a sagittal crest. The morphology of the fragment is anything but like *Homo erectus*. At the midpoint of the orbit, the temporal line is only 13.3 mm posterior of the anterior supraorbital margin⁹. In all crania of *Homo erectus*, a measurement from the midpoint of the orbit to the temporal line is impossible. The line becomes anterior-posterior without approaching the sagittal suture closely enough to be posterior of the orbit midpoint. The morphological difference shown by *Homo erectus* specimens in this region can even be seen in a very early cranium such as OH 16. In OH 16 the measurement is impossible, although the temporal lines approach within about 40 mm of each other. In comparison, the measurement is possible in STS 5 although the temporal lines never come closer than 60 mm.

(9) Clarke *et al.* considered that a parietal fragment, SK 847e, may belong to the composite cranium. The superior temporal line is some distance from the sagittal suture in this specimen, 37 mm at lambda and a maximum of 39 mm farther forward. Clarke *et al.* feel¹ this indicates a masticatory complex very different from what is typical at Swartkrans. If the association with the cranial-facial portion is correct, their conclusion does not necessarily follow. Robinson has clearly shown¹⁴ that the position of the temporal lines, and the presence of a sagittal crest, is a function of cranial size in the australopithecines, both gracile and robust, just as it is in gorillas and chimpanzees. Thus among the gracile australopithecines, only MLD 1 has a sagittal crest. MLD 1 has an occipital breadth of 85 mm and all other measurable gracile australopithecine occiputs are less than 80 mm in breadth. High temporal lines and ultimately sagittal crests occur in larger hominoid specimens within each taxon because dietary requirements, and concomitantly jaw size and robustness, increase at a rate faster than body size (the allometric coefficient is greater than 1). The same relation holds for robust australopithecines; smaller specimens are not as likely to have temporal lines as

high as larger ones. Table 1 gives data for the breadth across the mandibular fossae, approximating the bicondylar breadth of the mandible. This measurement of cranial size, as all the others discussed, is smaller than any other robust australopithecine in SK 80/846/847. Given the much smaller cranium, the low temporal lines do not necessarily indicate a difference in masticatory adaptation, any more than sagittal crested chimpanzee males discern a different dietary adaptation than other uncrested males or uncrested females.

(10) The parietal SK 847e could belong to a juvenile. Juvenile specimens such as SK 54 are known to occur at Swartkrans, with crania of almost adult size (the cranial capacity of SK 54 is about 500 c.c.¹⁵), but without sagittal crests^{9,15}. In SK 54, the closest approach of the temporal line to the sagittal suture is 38 mm⁹. The length along the sagittal suture is 82.5 mm in SK 847e¹. This distance is measured from lambda to the most anterior point. If bregma is not much farther anterior, this is a relatively short bregma-lambda distance for a robust australopithecine, let alone *Homo erectus*. The corresponding distance for SK 46 is at least 86 mm¹⁰, and for Koobi Fora 158 it is about 85 mm¹¹. The published measurement for OH 5 is 76 mm⁹. I think this is an underestimate. The facial-frontal fragment is nowhere continuous with the parietals. Reconstruction of OH 5 on the model of Koobi Fora 158, a more complete robust specimen, indicates a greater sagittal length for OH 5. Otherwise, the later cranium has a bregma-lambda distance 9 mm shorter, but a cranial length 13 mm longer. In fact, the published OH 5 bregma-lambda measurement is only 2 mm greater than that of the Taung child¹⁶ with an estimated age of 6 yr. Finally, C. L. Brace measured the bregma-lambda distance as 86 mm on the Tobias reconstruction of OH 5 at the National Museum, Nairobi.

(11) Tobias demonstrated⁹ the australopithecine character of the SK 847 supraorbital torus. It is the thinnest of all robust australopithecine tori, whereas the condition in *Homo*

erectus is quite different (Table 1). The supraorbital sulcus of the specimen does not appear different from the sulcus of SK 46, beside the Swartkrans composite in Fig. 3 of the original publication¹. If the temporal lines in the region of the orbits are more gracile in SK 847 than in other robust australopithecines, this would be expected from the smaller size of the specimen. Its resemblance in this area is with the australopithecines, and not with the known specimens of *Homo erectus*.

I believe that the total morphological pattern of SK 846/847, along with that of the maxilla SK 80 (discussed elsewhere^{5,17}), indicate the composite cranium is a small robust australopithecine. The differences between this cranium and the others at Swartkrans are far less than those between *Pan troglodytes* and *Pan troglodytes paniscus*¹⁸, although in both the hominid and the pongid the size variation gives similar morphological variation.

The confusion of *Homo erectus* specimens and robust australopithecines is not limited to Swartkrans. The close relation of these taxa has led to the identification of OH 16, the Chad facial fragment, and the "*Meganthropus*" mandibles as *Homo erectus* by some workers, and as robust australopithecines by others. It seems that there may be a similar disagreement with respect to Koobi Fora 210 (refs. 11 and 19).

In sum, SK 80/846/847 has the morphology that could be expected of a robust australopithecine cranium small enough to fit SK 74a.

I thank Dr C. L. Brace for the photographs of SK 74a and SK 12, and Mr R. J. Clarke, Dr C. K. Brain and Dr F. Clark Howell for the photograph of SK 80/846/847.

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Protection of Hermit Crabs (*Dardanus* spp.) from Octopus by Commensal Sea Anemones (*Calliactis* spp.)

PARTNERSHIPS between sea anemones and hermit crabs have long been considered as examples of commensal associations that seem to benefit both partners. In some of these partnerships the pagurid shows no behaviour pattern towards the anemone (*Pagurus bernhardus* (L.) and *Calliactis parasitica* (Couch)¹). In these associations the crab probably receives no benefit, all gain going to the anemone which, by clinging to the shell and transferring itself to it, has the advantage of being transported. In most cases, however, especially those involving *Dardanus* spp., the pagurids actively transfer their commensal anemones to their shells²⁻⁵; in these associations the crab could be expected to benefit in some way. Camouflage, assistance in capturing prey, protection from predators and cover for weak shells have all been suggested as possible gains for pagurids that carry anemones, but there has been little direct evidence to support any of these possibilities⁶.

Following up a suggestion of Dr Peter Dohrn, I have studied the behaviour of *Octopus vulgaris* when brought together with the two Mediterranean species of *Dardanus* (*D. arrosor* (Herbst) and *D. callidus* (Risso)) with and without their commensal anemones, *Calliactis parasitica*. Tables 1 and 2

Table 1 Results of Encounters between *Octopus vulgaris* and *Dardanus* without *Calliactis* on their Shells

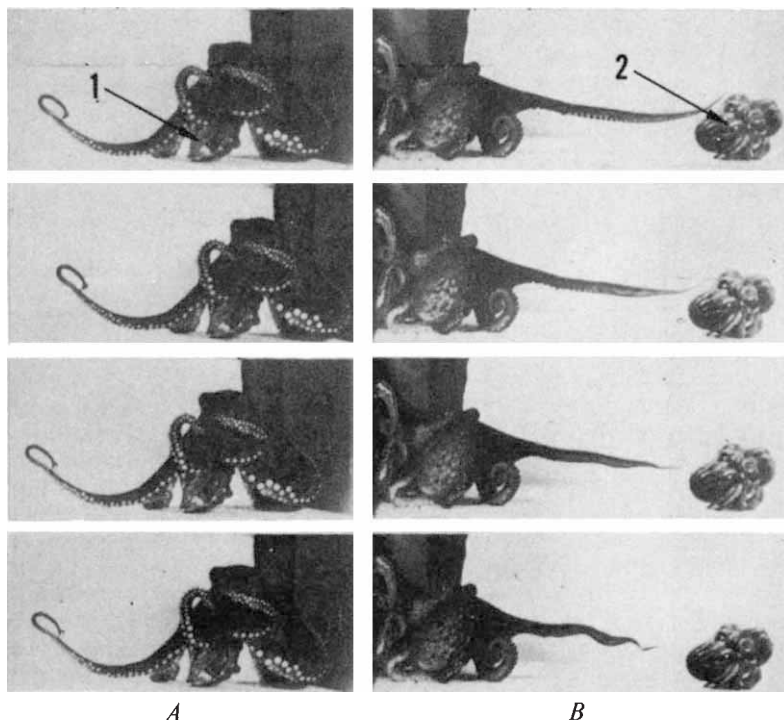
Octopus	Dardanus	First attack		No. of attacks			Final result
		Time (min)	Duration (min)	0-1 h	1-3 h	3-6 h	
1	<i>D. arrosor</i>	<1	5	2	7	3	Ingested after 89 h
2	<i>D. callidus</i>	35	1	1	5	2	Ingested after 38 h
3	<i>D. arrosor</i>	<1	15	1	1	3	Ingested after 41 h
4	<i>D. arrosor</i>	>360	—	Nil	Nil	Nil	Ingested after 66 h
5	<i>D. callidus</i>	<1	190	1	Continuous	Continuous	Ingested after 17 h
6	<i>D. callidus</i>	<1	20	2	Nil	2	Ingested after 29 h

Table 2 Results of Encounters between *Octopus vulgaris* and *Dardanus* with *Calliactis* on their Shells

Octopus	Dardanus	First attack		No. of attacks*			Final result
		Time (min)	Duration (s)	0-1 h	1-3 h	3-6 h	
1	<i>D. arrosor</i>	<1	5	10	4	5	Not ingested in 90 h
2	<i>D. callidus</i>	<1	15	2	1	2	Not ingested in 150 h
3	<i>D. arrosor</i>	<1	3	3	3	5	Not ingested in 71 h
4	<i>D. arrosor</i>	<1	10	14	9	7	Not ingested in 88 h
5	<i>D. callidus</i>	<1	5	4	1	2	Not ingested in 40 h
6	<i>D. callidus</i>	3	10	6	Nil	2	Not ingested in 65 h

* "Attacks" in these cases were mostly brief exploratory contacts.

Fig. 1 Two sequences from ciné film record showing: (A) *O. vulgaris* holding *Dardanus* without *Calliactis* and beginning to enclose in web before settling on top; (B) quick withdrawal of arm by *O. vulgaris* immediately after touching tentacles of *Calliactis* on shell occupied by *Dardanus*. *Dardanus* in (A) were eaten between 37 and 47 h; *Dardanus* in (B) survived until removed after 70 h and without attacks being seen after 8 h. Sequences (A) and (B) are four successive frames from 16 mm Ektachrome print taken at twenty-four frames per s (duration of each sequence 0.12 s). (1) *Dardanus* held by octopus; (2) *Calliactis* on shell occupied by *Dardanus*.



show results of six paired trials in which single crabs were introduced into tanks containing one *O. vulgaris*. In every case, the octopus attacked, usually at once. All *Dardanus* without *Calliactis* were eventually ingested (Table 1). None of those with *Calliactis* were ever taken (Table 2); in fact none was ever held by the octopus for longer than 15 s.

When there were no anemones present, attacks developed into attempts to dislodge the pagurid by inserting an arm into the aperture of the shell and then enveloping the shell and the withdrawn crab in the web (Fig. 1A). The octopus remained thus, probably pulling strongly, often for many hours. In one case (No. 1 in Table 1) the crab was extracted only after 3 days 17 h, many earlier attempts having been unsuccessful.

The first attacks on *Dardanus* with *Calliactis* on their shells always ended with *O. vulgaris* retreating, as if hurt, within seconds. Usually, the attack was repeated several times during the next few hours but eventually these attacks became nothing more than cautious approaches. In these encounters the octopus behaved as if it had received a punishing stimulus, especially if the tentacles of *Calliactis* were extended. On coming into contact with the tentacular crown, an octopus arm was always pulled back sharply as if stung (Fig. 1B). In the end, *Dardanus* with *Calliactis* moved about the tank freely without being molested. Indeed, after 24 h no encounters were seen and *O. vulgaris* often removed itself to the top of the tank when the crab approached.

Six other trials were carried out (episodes from three of these were recorded on 16 mm film, from which two excerpts are shown in Fig. 1) in which only the final outcome was noted. These confirmed that *Dardanus* with *Calliactis* on their shells were completely protected from *O. vulgaris* which earlier had invariably taken anemone-free *Dardanus* in comparable situations. Similar trials with *Pagurus prideauxi* and *Adamsia palliata* showed that this pagurid was not protected by its anemone. Not being able to withdraw completely (the anemone and the shell barely cover the abdomen), all *P. prideauxi* were ingested after single attacks.

There were no signs that *O. vulgaris* suffered any ill effects from its brief encounters with *Calliactis*. This was not the case when *P. prideauxi* were being ingested with *A. palliata* still in position on the crab. Then, after beginning to envelop the pair, the arms and the web of the octopus withdrew, curling upwards and out of contact with the anemone, revealing an

immobilized crab held in the jaws. One octopus remained thus quite still for 25 min. During this time the skin was extremely pallid, the rate of mantle beating rose above 1 per sec (normally about 1 per 2 s) and on the ventral surface of the mantle many small blisters appeared. But when the crab was eventually extracted and the *Adamsia* could be discarded, *O. vulgaris* soon took on its normal behaviour and appearance.

The distributions of various species of *Dardanus* and *Calliactis* follow closely the distribution of *O. vulgaris* around the world. All are essentially warm temperate to tropical species occurring sublittorally in the Mediterranean, the Eastern and South Atlantic, the Caribbean, the Indian Ocean and the Western and Central Pacific⁷⁻⁹. Indeed, *O. vulgaris* has been reported in almost all those locations where *Dardanus* and *Calliactis* are common. In the Mediterranean, *Dardanus* inhabits sandy bottoms with some exposed rocks (*D. calliactis* more often on rocks than *D. arrosor*) from sublittoral to moderate depths down to 100 m where *O. vulgaris* also occurs.

It seems therefore that by carrying *Calliactis* on its shell, *Dardanus* is protected, presumably by the nematocysts of the anemones, from a creature that otherwise would be a most effective predator.

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Copper Tolerance in *Becium homblei*

A ZAMBIAN flowering plant *Becium homblei* of the family labiatae grows on soils rich in copper¹, and it has been shown to accumulate heavy metals²⁻⁴. Other plants also have this characteristic, and their ability to immobilize in insoluble form and to regulate internal distribution of absorbed metals has been noted^{5,6}. We agree that these two properties are important in *B. homblei*, but also suggest that an extrinsic factor, peculiar to the derived savanna vegetation of south-central Africa, must be taken into account.

B. homblei is a small plant which forms a clump of unbranched stems, about 18 inches high, from a perennial rootstock. Stems and leaves usually appear early in September before the end of the dry season. Young leaves are dark green, though a few may be yellow. The number of chlorotic leaves increases, until by the end of the growing season (April or May) 60% of the plants may show signs of chlorosis. The leaves wither in the dry season and stems and leaves are usually burned away in bush fires.

Analyses show that *B. homblei* has apparently no mechanism for limiting copper uptake. As growth proceeds, the concentration of metal increases in leaves and stems. We have recorded, during 1 month, an average increase of 75% in total leaf copper. We have also shown, by paper and thin-layer chromatography, that none of this copper is ionic but exists in complexed forms. Fractionation of plant tissue into extractable-free, detergent residue and H₂SO₄-insoluble residue, represent-soluble, cell wall and lignin fractions^{7,8} indicates that much of the copper is bound to structural material of the cells. As is also seen from Table 1, the difference between normal and chlorotic leaves seems to lie not in total copper content, but in the amount extractable in water and organic solvents. There is, moreover, a significant difference between the amount of extractable material in root and leaf tissues. We suggest that these differences, in conjunction with the extrinsic factor of regular bush fires, were important factors in the evolution of this copper-resistant species of *Becium*.

If roots of *B. homblei* are cut in the dry season, they ooze a considerable amount of sap which undoubtedly permits new growth to take place before the rains begin. This root water contains little copper, and thus copper toxicity is unlikely to occur in new leaves. When the rains begin, copper contaminated water passes from the soil to stems and leaves. Much of the copper is rendered insoluble, but enough remains in solution to produce toxic effects. Thus chlorosis develops with the increasing uptake of copper. With leaf fall and subsequent burning, the plant is freed from the bulk of its accumulated

copper. Thus, at the beginning of the new growth season, the plant develops new leaves and produces flowers and seeds, before it is affected by the copper.

When stems are clipped from a clump of *B. homblei* in the dry season and early sprouting is induced by watering the roots, the new leaves are nearly always chlorotic. This phenomenon is apparently caused by contaminated water reaching the developing leaves. When, as sometimes happens, some stems survive the dry season and are protected from fire, the new leaves which sprout from them are usually chlorotic also. Again, this could be explained on the assumption that water supplied from the root, though initially non-toxic, is contaminated with soluble copper on its way through the stems.

As Bradshaw *et al.*⁹ wrote of adaptation to metal toxicity in *Agrostis tenuis*, it is tempting to believe that purely Darwinian (selective) processes are insufficient to explain the phenomenon, and that the tolerance is Lamarckian (acquired) in origin. In the light of such a surmise, it would be interesting to see whether prolonged protection of *B. homblei* from the external influence of fire would finally result in the complete disappearance of the plant as a result of the uninterrupted accumulation of toxic concentrations of copper in leaves and stems.

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Estimation of Ecological Production where Age of the Population is Unknown

HITHERTO methods of production estimation¹ have presupposed the estimation of the age of animals, from population data, from laboratory observations on growth rates, or from periodic marks found in hard structures of the body². Frequently no such method is suitable and it becomes impossible to estimate production.

An estimate of production (P) can be derived from knowledge of G , the instantaneous growth rate³, and the mean biomass, $\bar{B}$:

$$P = G\bar{B} \quad (1)$$

This assumes that G is constant for individuals of different sizes and ages; errors will be small if production is calculated for separate size or age classes, whose width is small. We shall calculate production for 1 yr, the time interval most frequently used by fishery biologists. P may also be estimated graphically from Allen curves⁴.

The value of G decreases with age unless animals enter a new growth stanza⁵, or stop growing altogether. Greze⁶ has related variation of G to percentage final body weight (%FBW). This procedure has the advantages of excluding consideration of absolute body weight and of permitting us to consider relations between relative size and growth rate. Greze used

Table 1 Distribution of Copper in Fractions of Leaf and Root of *B. homblei*

	Copper (mg/kg dry wt.)		Root
	Normal	Chlorotic	
Total	20.2 ± 5.8	16.6 ± 1.4	9.9 ± 2.2
Extractable-free	12.2 ± 3.9	5.8 ± 1.0	7.9 ± 1.2
% of total extracted by water/organic solvents	40	65	20
Acid detergent residue	4.5 ± 0.9	4.0 ± 1.3	2.6 ± 1.8
% of total extracted by detergent	37	11	50
H ₂ SO ₄	1.3 ± 0.5	0.6 ± 0.6	0.9 ± 0.4
% of total extracted by H ₂ SO ₄	16	20	22

Results are related to original dry tissue and are the mean of five observations ± standard deviation. The figures given for the amount of copper extracted by the different treatments are expressed as the approximate percentage of the total copper originally present in the tissue. The acid detergent was 1% (w/v) cetyl trimethyl ammonium bromide in 1 N H₂SO₄ and extraction was for 2 h. Extractable-free residue was prepared by exhaustive refluxing with water and ethanol: benzene (1:2, v/v) mixture. H₂SO₄ residue was what remained after overnight treatment of acid detergent residue with 72% (w/v) sulphuric acid.

a complex hyperbola of the form:

$$y = 0.0026 - 0.000024(\%FBW) + \frac{0.0151}{\%FBW}$$

where y is relative daily weight increment (an approximation of G on a daily basis).

Because G is known to vary with age it seemed possible that the length of an animal's life cycle—its cohort duration in years, n —might also influence the variation of G . The relations of age, annual G and $\%FBW$ were examined for twenty-nine populations of fish, crustacea and molluscs⁸⁻²¹. Rectangular hyperbolae were fitted by least squares regression using equations of the form

$$G = a + \frac{b}{\%FBW} \quad (2)$$

The data were (i) grouped according to cohort durations, (ii) treated as a single group regardless of cohort duration (Table 1). All lines are highly significant: above values of 15–20% FBW , 95% confidence limits are less than $\pm 10\%$ of the mean. The regression coefficient of the grouped data decreases with increasing cohort duration (Table 1, Fig. 1).

Differences between the group lines (a – e) were tested by comparing the residual sums of squares for the joint fit (f) with the residual sums of squares for the five individual fits. The F test shows that equation (f) gives a distinctly worse fit to the data than equations (a) to (e). We must therefore conclude that the form of relationship (2) depends on cohort duration. For the purposes of approximation, the loss of information incurred by ignoring age (line f) was calculated as 13% (Table 1).

The joint line introduces a small error compared with that usually found in population data, and so it may be used to calculate production for populations of unknown age composition. Length frequency distributions of twelve monthly samples of bleak (*Alburnus alburnus* L.) and dace (*Leuciscus leuciscus* L.) were used to test this method²². The values of G at each $\%FBW$ are obtained from equation (f). Multiplying each G by the biomass of the size interval corresponding to it gives the production of each size class. Table 2 shows these steps for bleak (method 1). Estimated production was

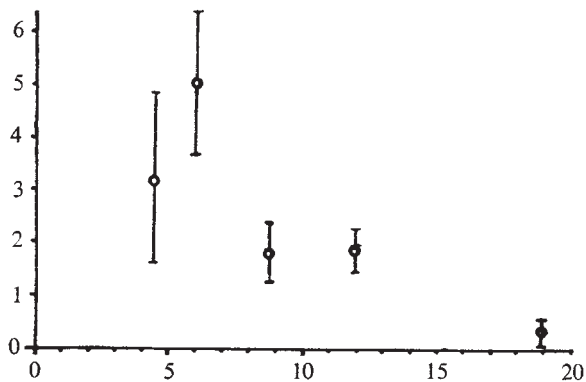


Fig. 1 Ordinate: regression coefficients in Table 1. Abscissa: cohort duration, n , in yr. Means and 95% confidence limits are shown.

9.3 g/m²/yr compared with 12.3 g/m²/yr calculated from Allen curves⁷ for fish which correspond to the size range examined. Corresponding values for dace are less close: 0.46 and 0.30 g/m²/yr respectively. As the census data used in constructing the Allen curves have 95% confidence limits of $\pm 50\%$ of the mean, these agreements must be regarded as reasonable.

An alternative method of investigation is to consider the relationship between G and body weight (BW):

$$G = a + \frac{b}{BW} \quad (3)$$

for each species separately and to try to discover patterns in the fitted values of a and b .

Equation (3) was fitted to data from eleven species. The values of a are fairly consistent over species, while b increases with the size of the species. Regressions of the form of equation (3) with a held constant over the eleven species gave a fit no worse than the individual lines (the fitted value of the common a is 0.295). The eleven values of b were plotted against the estimates of FBW on log scales (Fig. 2). The best fitting regression of $\log b$ on $\log FBW$ has a slope of 1.10, which is not significantly different from 1.00. The fitted line

Table 1 Relationships between Annual Instantaneous Growth Rates and % Final Body Weight

Distinguishing symbol	Cohort duration (n)	No. of observations	Equations	s.e.	r	Value of P on F test
(a)	3-5	30	$G = 0.262 + \frac{3.158}{\%FBW}$	± 0.770	0.511	0.01
(b)	6	21	$G = 0.123 + \frac{4.993}{\%FBW}$	± 0.770	0.831	0.001
(c)	8-10	49	$G = 0.260 + \frac{1.790}{\%FBW}$	± 0.264	0.703	0.001
(d)	11-13	77	$G = 0.162 + \frac{1.867}{\%FBW}$	± 0.200	0.733	0.001
(e)	19	57	$G = 0.204 + \frac{0.351}{\%FBW}$	± 0.061	0.613	0.001
(f)	All	234	$G = 0.261 + \frac{0.519}{\%FBW}$	± 0.458	0.210	0.001

(i) Sum of residual sums of squares for (a)–(e): 8.355 d.f. 224
(ii) Residual sum of squares for (f): 9.861 d.f. 232
(i)–(ii): 1.506 d.f. 8
 $F = 4.277$ at 8 and 224 d.f., 0.001 P
(iii) Fitted sum of squares for (f): 11.57
loss of information: $\frac{1.506}{11.57} = 0.1301$

Table 2 Data for Production Estimation of Bleak

Size class (fork length cm)	7	8	9	10	11	12	13	14	15	16	Totals
Body weight (wet g)	2.95	4.67	6.92	10.0	14.7	20.0	26.3	32.4	—	51.1	
Total Nos. caught/100 m ²	Accurate data not available			158.8	82.85	19.29	2.59	0.49	—	0.09	264 (3+ and older)
Biomass (g/100 m ²)	Accurate data not available			1,558	1,218	385.8	68.1	15.9	—	4.6	
%FBW	5.76	9.12	13.5	19.5	28.7	39.1	51.4	63.3	—	100	
g (g/g/yr) (1)	0.351	0.318	0.299	0.288	0.279	0.274	0.271	0.269	—	0.266	
(2)	0.787	0.605	0.505	0.440	0.394	0.368	0.350	0.340	—	0.323	
Production (1)	Accurate data not available			457.3	339.8	105.7	18.5	4.3	—	1.2	926.8 g/100 m ² /yr
(g/100 m ³ /yr) (2)	Accurate data not available			698.7	479.9	142.0	23.8	5.4	—	1.5	1,351.3 g/100 m ² /yr

(1) First method; (2) second method, where these differ.

with a slope of 1 is:

$$\log b = 1.55 + \log FBW$$

Substituting for b in equation (3) gives:

$$G = a + \frac{0.0283 FBW}{BW}$$

$$G = a + \frac{2.83}{\%FBW} \quad (4)$$

(Note the similarity to the fitted lines in Table 1.)

Fitted lines for a single species in different habitats or at different times would show whether the intraspecies variation of b takes the same form as the interspecies variation. The regressions were compared for different times for whitefish¹³ and for different habitats for cod¹². The values of b and FBW for these two groups of data are shown in Fig. 2 where they appear as a fairly random scatter. More detailed investigation shows that b decreases consistently over the years that whitefish were sampled. For cod, b increases with increasing W longitude and with decreasing N latitude. In spite of this variation it seems possible to estimate b from FBW and so to obtain estimates from equation (4). The results for bleak are shown in Table 2 (method 2); estimated production is 13.5 g/m²/yr, which is reasonably close to the estimate from Allen curves: 12.3 g/m²/yr. For dace the results are less close: 0.54 and 0.30 g/m²/yr.

A third method of estimating production is applicable when data from a capture-recapture study to estimate population density are available. If the growth of some individuals marked in the initial sample can be estimated when the individuals are recaptured, a relationship between growth over the intervening period and size at the first capture can be obtained.

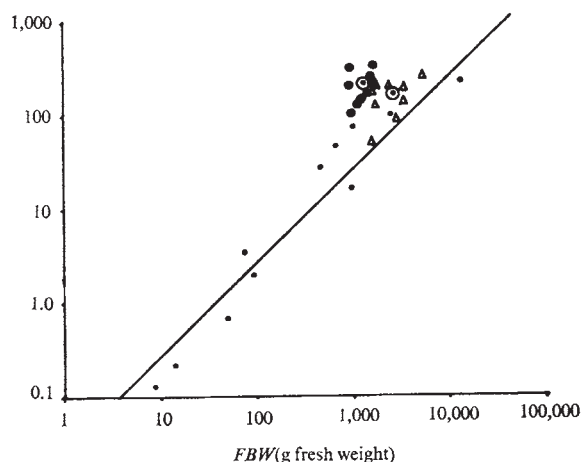


Fig. 2 Variation of b (equation (3)) with FBW for thirteen species. Single observation. Mean of several observations. Whitefish, different years. Cod, different habitats.

The production in each size class can be estimated from the number in that class at the first capture and the growth rate estimated from the fitted relationship. The numbers in each size class must be determined by some other technique, such as catch per unit effort. The only assumption necessary for this method is that the probability of recapture of a particular fish and its growth over the intervening period are not related, which seems unexceptionable. Size selective mortality will only reduce the information in the corresponding part of the growth- BW curve. An important point to note about this method is that there is no need to estimate FBW .

All three methods are in principle applicable to animals in which indeterminate growth occurs. The third method should be applicable to a population without reservations where a clear relation between G and BW exists. For the first two methods to be used it must be assumed that the population examined shows the same growth characteristics as the populations studied here.

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Effects of Ethirimol on Cytological Characters in Barley

THERE is increasing evidence that chromosome size and mass are highly variable within a species. Both natural and induced variation in the DNA^{1,2} and protein³⁻⁵ content of chromosomes is known, and I have demonstrated a positive linear correlation between naturally occurring changes in the volume of metaphase chromosomes and the RNA and histone protein content of the nuclei of *Vicia faba*. The changes in chromosome volume were not accompanied by variation in the amount of 4C DNA. Similar changes are known to be induced by variations in the mineral content of a culture solution⁵, in temperature⁷ and in the hormone balance of plants (personal communication from N. Jones). It has been suggested^{5,6} that such changes within a species are correlated with the metabolic activity of the meristems in which they occur. To test this hypothesis, I have investigated the effects of ethirimol, which is known to affect root growth, on chromosome volume and root length. If the hypothesis is correct, ethirimol should affect both characters in the same way. Apart from the theoretical interest of such a study, it seemed useful to investigate any possible cytological effects of ethirimol, which may be used widely as a fungicide.

Ethirimol⁸ (5-butyl-2-ethylamino-4-hydroxy-6-methyl pyrimidine) or 'Milstem' (ICI) is a white crystalline solid with a water solubility of 0.2% at room temperature. It acts as a systemic fungicide and is particularly effective against powdery mildew of barley (*Erysiphe graminis* DC. f. sp. *hordei* Marchal). When ethirimol is applied as a seed dressing it is released slowly from the soil during a long period so that small amounts are continually present in the plant. Such application thus gives long lasting control of mildew from germination until ear emergence. The fungicide is translocated in the xylem but not in the phloem and at normal rates of application is claimed not to be phytotoxic.

Three varieties of barley (*Hordeum vulgare*)—'Julia', 'Sultan' and 'Maris Otter'—were grown either in culture solution or in the field. In culture experiments seeds were germinated on moist filter paper and the resulting plants were transferred after 3 days to aerated modified Hoagland culture solution so that their roots were immersed. Ethirimol was added as a wettable powder to the culture solution and renewed weekly so that the concentration of ethirimol available to the plants throughout their life history did not vary. In the field experiment the concentration of ethirimol available may have varied widely during the life of the plant because the fungicide was applied only once, as a seed dressing.

In the first experiment 'Julia' plants were grown in culture solution with or without 16 p.p.m. of ethirimol in a glasshouse at 20° C with continuous light. After 2 weeks plants grown in the presence of ethirimol had much shorter roots than those grown without the fungicide, but there was no significant difference in shoot length (Table 1).

Chromosome volume was measured in well spread somatic metaphase plates in root tip squashes made from new root, less than 1 cm long. The total chromosome volume per cell in plants grown in the presence of ethirimol was 68% of that in plants grown without the fungicide (Table 1)—a highly significant difference. The observation that decreased root growth is accompanied by a decrease in chromosome volume

Table 2 Mean Chiasma Frequency

Experiment	Ethirimol	'Julia'	'Sultan'	'Maris Otter'
1	0	13.91 ± 0.03		
	16 p.p.m.	13.35 ± 0.08		
	0	13.88 ± 0.03	13.92 ± 0.02	
2	0.16 p.p.m.	13.92 ± 0.02	13.78 ± 0.02	
	16.0 p.p.m.	13.51 ± 0.03	13.64 ± 0.05	
3	0			13.71 ± 0.03
	4 pounds/acre			13.46 ± 0.03

supports the hypothesis that changes in the volume of metaphase chromosomes reflect the level of metabolic activity of the meristems in which they occur. The decrease in chromosome volume in roots treated with ethirimol does not necessarily indicate a specific effect of the fungicide on this nuclear character, but it probably reflects a toxic effect of the chemical on the growth rate of treated roots. It should be emphasized that a treatment of 16 p.p.m. of ethirimol is extremely large compared with the rate of application recommended by ICI. When the fungicide was supplied in a concentration of 0.16 p.p.m.—much closer to the maker's recommendation—root and shoot growth increased.

Plants in the first experiment were grown until heading, after 10–11 weeks, and suitable inflorescences were then fixed. Table 2 shows that the fungicide significantly reduced chiasma frequency in pollen mother cells when plants were free from infection by powdery mildew throughout their life history.

In a second experiment 'Julia' and 'Sultan' barley plants were inoculated with powdery mildew (race C5, isolate B8 104) which attacked 'Julia' but not 'Sultan'. Plants of both varieties were grown in culture solution containing 0, 0.16 or 16 p.p.m. of ethirimol. Chiasma frequency in pollen mother cells was scored (Table 2). Both concentrations gave complete control of powdery mildew, although when no ethirimol was used only 'Julia' was infected with the fungus. There was no significant difference between varieties, and no interaction between treatments and varieties; the only significant difference was between treatments. There was a decrease in mean chiasma frequency in the pollen mother cells of plants treated with 16 p.p.m. of ethirimol, compared with untreated plants, whether infected with powdery mildew or not.

When, in the third experiment, 'Maris Otter' was grown in the field with 0 or 4 pounds/acre of ethirimol added as a seed dressing, there was again a small but highly significant reduction in chiasma frequency in the pollen mother cells of treated plants compared with untreated plants (Table 2). In this case the rate of application was four times greater than that recommended by ICI⁸.

The small reduction in chiasma frequency, even when ethirimol is supplied in large concentrations, indicates that losses in yield due to infertility are most unlikely as a result of fungicide treatment. Plant breeders and cytologists should, however, use ethirimol cautiously on experimental material if chiasma frequency is to be determined and on early generations of genetically segregating material.

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Table 1 Mean Length of the Longest Root and the Shoot and Chromosome Volume of 'Julia' Barley 2 Weeks after Germination

Ethirimol (p.p.m.)	Root (cm)	Shoot (cm)	Chromosome* volume (μm ³)
0	20.75 ± 0.52	20.89 ± 0.59	185.1 ± 2.36
16	10.98 ± 0.20	19.78 ± 0.37	126.4 ± 3.05

The decrease in mean chiasma frequency in treated plants was between 2 and 4% in each case.

* Figures are the mean values for ten cells.

Pneumococci insensitive to Penicillin

PNEUMOCOCCI of all capsular types have been regarded as highly sensitive to penicillin¹ since its introduction for the treatment of human infections in 1940. Although penicillin-insensitive mutants can be selected *in vitro* by repeated sub-culture in the presence of sub-inhibitory concentrations of penicillin²⁻⁶, resistant wild strains were not recognized until 1967, when penicillin-insensitive pneumococci (type 23) were isolated from a patient with hypogammaglobulinaemia and bronchiectasis, who had received much antibiotic therapy, including penicillin⁷. We now report further strains of pneumococci, isolated in Australia and New Guinea, relatively insensitive to both penicillin and cephalosporin antibiotics.

These penicillin-insensitive pneumococci were isolated from an aboriginal child at Ernabella, South Australia, in 1967, and from fifteen New Guineans at Anguganak in the West Sepik district during a fourteen week period in 1969. Pneumococci were typed by the specific capsular reaction, using typing sera from the Statens Seruminstitut, Copenhagen, and the Danish system of naming types has been followed. The pneumococcus from the aborigine, which was isolated from the upper respiratory tract, was identified as a type 6 strain. Most of the Sepik isolates were also from carriers, but two were isolated from subjects receiving penicillin for respiratory infections: all were identified as type 4.

The penicillin-insensitive strains were Gram-positive cocci with the morphology of pneumococci; cultural characteristics and α -haemolysis on horse blood agar were also typical; bile solubility and sensitivity to ethyl hydrocupreine hydrochloride ('Optochin') were demonstrated. Diffuse turbidity was produced in horse serum broth and long chains, typical of rough pneumococci, were not formed. Capsules were readily demonstrated by the specific capsular reaction. When inoculated by the intraperitoneal route, both type 4 and type 6 strains were virulent for white mice, an inoculum of 100 viable units or less producing a fatal infection.

Disk diffusion sensitivity tests showed resistance to penicillin but sensitivity to ampicillin, tetracycline, chloramphenicol, erythromycin, lincomycin and also to sulphadiazine and trimethoprim. When tested by the radial streak method, using as inoculum a standard 2 mm loopful of a 4 h culture in serum broth, growth occurred to within 2 mm of a disk containing 1 U (0.6 μ g) of penicillin G, whereas sensitive pneumococci tested as controls showed a zone of inhibition of 6 to 7 mm. Quantitative sensitivity tests were carried out by the plate titration method with horse blood agar, using antibiotic solutions which had been freshly prepared. Nine strains, including the Ernabella strain, were tested. The results (Table 1) indicated that the Ernabella and Anguganak isolates were uniformly insensitive to penicillin G, methicillin and cephalosporin antibiotics. Penicillin sensitive pneumococci of 16 different types (including types 1, 2, 3, 4 and 6) tested as controls were all inhibited by 0.02 μ g of penicillin G/ml.

The minimal inhibitory concentrations of penicillin and methicillin were 25 to 50 times that required to inhibit the sensitive pneumococci used as controls. There was also a significant degree of resistance to cephaloridine and cephalothin. It may be noted that the penicillin-insensitive variants of pneumococci described by Gunnison *et al.*⁶ were also insensitive to cephalothin. Quantitative tests by the plate

titration method usually showed sharp end-points, indicating that populations of these isolates were largely homogeneous in their resistance to penicillins and cephalosporins.

Pneumococci resistant to sulphonamides were first encountered in 1940, 5 yr after the introduction of these drugs. Resistance appeared in many pneumococcal types and there was cross-resistance, so that pneumococci resistant to one sulphonamide were resistant to all. But resistance usually occurred only in strains isolated from subjects who had prolonged treatment with sulphonamides.

For many years it was assumed that pneumococci were invariably sensitive to those antibiotics which are effective in coccal infections. In 1963, however, strains resistant to tetracycline were recognized⁸⁻¹¹. Such pneumococci are virulent for man, causing pneumonia and meningitis.

It may seem surprising that antibiotic-insensitive pneumococci should appear in a remote area such as the Sepik district, but much penicillin is used because respiratory and other infections are common in New Guinea. During the preceding 10 yr, the 507 inhabitants of the two villages at Anguganak had received 1,357 courses of procaine penicillin, which represents, on an individual basis, a course of penicillin every 3.7 yr. Moreover, during campaigns to control yaws, penicillin was administered to the entire population. Additional factors may be a high pneumococcal carrier rate, especially in children, and the heavy colonization of such carriers (ref. 12 and unpublished results). The isolation of the insensitive type 4 strain from fifteen individuals suggested that transmission had occurred, as these subjects lived in two nearby villages. This is analogous to cross-infection with tetracycline-resistant pneumococci which has occurred within hospitals^{10,13}.

In all other respects than antibiotic sensitivity, the penicillin-insensitive isolates have the characteristics of encapsulated pneumococci (results presented at the annual meeting of the Australian Society for Microbiology in 1970). Pneumococci of these types, 4 and 6, commonly cause infections in man, and epidemics of pneumonia due to type 4 pneumococci have been reported.

At present, the incidence of penicillin-insensitive pneumococci in Australia seems to be low: during 1965 to 1969 inclusive 1,242 smooth pneumococci were examined by a standard technique for sensitivity to penicillin G and other antibiotics (unpublished results of D. H.). Penicillin-insensitive strains from two subjects were detected during this period, an incidence of only 0.2%. The results of a preliminary examination of pneumococci from several districts in New Guinea suggest that the incidence of insensitive strains is greater than in Australia; studies of the distribution and frequency of such resistant strains are in progress.

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Table 1 Quantitative Antibiotic Sensitivity Tests with Insensitive and Control Pneumococci

	Minimal inhibitory concentration (μ g/ml.) Test strains	Control strains	Resistance ratio
Penicillin G	0.5	0.02	25
Methicillin	5 to 10	0.2	25 to 50
Ampicillin	0.1 to 0.2	0.05	2 to 4
Cephaloridine	0.5	0.05	10
Cephalothin	2.0	0.1	20

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Antischistosomal Action of Tubercidin administered after Absorption into Red Cells

SCHISTOSOMES seem to depend primarily if not exclusively on the anabolism of preformed purines for their total purine nucleotide requirements (ref. 1 and our own unpublished observations). Among various purine analogues we tested against *Schistosoma mansoni* *in vitro*, tubercidin (7-deazaadenosine)² was the most active, causing early separation of paired adults, alterations of the muscular activity pattern, and inhibition of egg laying in the medium when present in a concentration as low as 10^{-7} M. When administered to *S. mansoni*-infested mice either intraperitoneally or orally, tubercidin in effective dose regimens invariably caused 20–30% mortality. In an attempt to increase the margin of safety of this potentially useful antischistosomal agent, we considered the following facts in planning a new strategy of treatment. Schistosomes feed on blood cells, beginning about 2 weeks after cercarial penetration of the host³. Tubercidin is rapidly absorbed into red cells *in vitro* and is sequestered intracellularly in a phosphorylated form⁴. From 0.2–0.4 mg of tubercidin/ml. of whole blood can be so sequestered, and the longevity of tubercidin-containing red cells is unaffected⁴. We have investigated whether host red cells could deliver tubercidin to haematophagous forms of schistosomes, thereby increasing the selective toxicity of this antibiotic.

Young Ha/ICR female mice (14–16 g) were infested with *S. mansoni* by intraperitoneal injection of approximately 100 cercariae, collected from shedding snails (*Biomphalaria glabrata*, Puerto Rico strain 2). Beginning 2 weeks after infestation and then at weekly intervals for a month, mice in groups of ten were individually numbered, and 0.5 ml. of whole blood (approximately 30% of the total blood volume) was withdrawn from each mouse by the retro-orbital bleeding technique of Riley⁵. The heparinized aliquot of whole blood was mixed with 0.4 ml. of tubercidin solution (1 mg/ml. saline), incubated with gentle shaking at 37° C for 1 h, and then centrifuged at 1,000g for 5 min. The supernatant, containing any unabsorbed tubercidin, was aspirated and replaced by saline at 37° C. After the cells had been gently resuspended, they were returned to the original donor by way of a tail vein. Tubercidin (lot No. 8458-THP-65.5) was provided by Dr G. B. Whitfield, jun., of the Upjohn Co., Kalamazoo, Michigan.

All mice were killed 7 weeks after infestation and a determination was made of the number and condition of the schistosomes in the mesenteric veins, portal vein, and liver. A group of ten untreated mice, infested at the same time as the treated groups, served as controls. A 2 cm section of ileum was removed from each mouse for determination of the amount of egg deposition and the oogram pattern⁶.

The admixture of tubercidin-containing red cells with the residual blood cells *in situ* was timed to coincide with the various developmental stages of the parasite following cercarial penetration of the host. Thus, according to Clegg³, about 2 weeks after infestation surviving schistosomes have begun to feed on blood cells; by about the third week the reproductive organs have begun to form; by the fourth week gametogeny

has been completed and mating has begun; by the fifth and sixth weeks egg-laying has commenced and full maturity has been reached. The longevity of mouse red cells is approximately 3 weeks⁷, so that even the most immature schistosomes in this study had an opportunity to ingest tubercidin-containing red cells up to the time when the females would ordinarily begin to lay eggs.

The viability and egg-laying capacity of schistosomes in all the treated mice were adversely affected (Tables 1 and 2). Certain differences in response to treatment, however, were noted between sexually mature and immature worms. Worms which were mature at the outset of treatment (groups 5 and 6) exhibited a more pronounced liver shift than did the immature worms (groups 2, 3 and 4), which may simply be due to the shorter interval between the time treatment was started and the time chosen for examination. Furthermore, although egg laying was suppressed in all groups, the oograms of mature worms revealed a large increase in the number of mature and dead eggs, while the oograms of immature worms indicated that a few females had recently recovered and had begun to deposit viable eggs only a few days before the end of the experiment.

The livers of mice harbouring immature schistosomes at the outset of treatment contained a few egg granulomas and lesions containing dead worms, but otherwise their colour and texture closely resembled those of normal livers. By contrast, the livers of mice harbouring mature schistosomes at the outset of treatment contained many egg granulomas and lesions containing dead worms. They were darker and more fibrotic than the livers of mice in the other treated groups.

The surviving schistosomes in all treated mice were smaller than the schistosomes in the control group and were pale and sluggish. More single males and females were found in the livers of mice harbouring mature schistosomes at the outset of treatment (Table 1).

Table 1 Effect of Tubercidin, administered after Absorption into Red Cells, on Viability and Distribution of *Schistosoma mansoni* in Mice

Time of single treatment (weeks post-infestation)	No. of mice surviving/10 post-infestation	Distribution of schistosomes 7 weeks after infestation (Average number of pairs: single males; single females)		
		Mesenteric veins	Portal vein	Liver
No treatment	10	33 ± 4; * 3; 0	3; 0; 0	2; 3; 0
2	9	7 ± 0; 2; 0	1; 0; 0	2; 2; 1
3	9	5 ± 1; 1; 0	2; 0; 1	3; 0; 2
4	10	5 ± 1; 3; 0	2; 0; 0	2; 1; 1
5	9	2 ± 0; 3; 0	2; 1; 0	4; 8; 2
6	7	0; 3; 1	0; 2; 0	4; 7; 6

* Standard error of the mean.

Table 2 Effect of Tubercidin, administered after Absorption into Red Cells, on Egg Laying and Oogram Pattern of *Schistosoma mansoni* in Mice

Egg deposition in 2 cm sections of ileum 7 weeks after infestation								
Time of single treatment (weeks post-infestation)	No. of positive mice/No. of survivors	Average No. eggs per positive ileal section	% in stages of embryonation					
			1st	2nd	3rd	4th	Ma-ture	Dead
No treatment	10/10	600	16	22	23	9	28	2
2	3/9	46	33	47	15	2	3	0
3	0/9	0	0	0	0	0	0	0
4	1/10	6	100	0	0	0	0	0
5	9/9	50	0	0	0	0	15	85
6	7/7	372	0	0	0	1	53	46

The incidence of mortality of infested mice following treatment was variable (Table 1). It is possible that such deaths were caused by the direct action against the host of tubercidin which after its absorption into red cells is slowly released into the plasma over a long period⁸. Smith *et al.*⁴ found that human blood cells are saturated with tubercidin at a concentration of 0.2–0.4 mg/ml. whole blood. On this basis, we injected intravenously between 0.1 and 0.15 mg of tubercidin (sequestered in red cells) into each mouse, about 4 mg/kg body weight. After a single intravenous injection, the LD₅₀ of tubercidin in mice is 35 mg/kg, but the maximum tolerated repeated intravenous dose (in rats) is less than 0.25 mg/kg daily for 19 days⁹. If the repeated-dose toxicity of tubercidin in mice is similar to that in rats, about 5–6% of the sequestered tubercidin would have to be released daily to achieve plasma levels that were potentially lethal for the host. This possibility remains to be explored. None of the surviving mice showed signs of drug toxicity at any time during these experiments, and the highest incidence of mortality occurred in the group with the most advanced disease (and the most damaged liver) at the time of treatment.

The possibility that the experimental protocol rather than tubercidin *per se* was responsible for the observed antischistosomal effects can be discounted, for identical procedures were followed in assessing the activity of adenine and 6-mercaptopurine arabinoside against *S. mansoni* in mice, and no deviations from control values were observed in any of the treated groups.

The efficacy of tubercidin against schistosomiasis caused by *S. mansoni* in mice, administered after absorption into red cells, can be considered an example of the exploitation of specific parasite mechanisms not shared by the host. Perhaps future studies concerned with the delineation of tubercidin's mode of action against schistosomes will lead to the development of related compounds with wider margins of safety.

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species culture. The constants α and β , the coefficients of competition, denote the relative effect of the abundance of one species on the population growth rate of the other. These relationships imply that for a stable competitive equilibrium to obtain it is necessary that

$$\alpha < \frac{K_1}{K_2}, \beta < \frac{K_2}{K_1}$$

Ayala³ raised two species of *Drosophila* in single and double species cultures and found that in certain conditions the two species persist together, apparently at equilibrium. He ran two competition series and measured all the K s and N s directly. From these he calculated the pairs of α s and β s. In both series

he found, contrary to theoretical prediction, $\alpha > \frac{K_1}{K_2}$ and $\beta > \frac{K_2}{K_1}$. That is, the total number of flies in the competition bottles was far lower than that predicted by theory.

Because the experiments did not confirm the predictions Ayala rejected the theory as false. But the equations that he applied to his experiments are meant to describe a competition situation in which a stable equilibrium obtains, whereas a close analysis of Ayala's data shows that there was no stable equilibrium in his experiments.

I examined his data (his Figs. 1 and 2) for the weeks 6–23 and 29–40 when the populations were supposed to be in equilibrium and looked at the changes in proportion of *D. serrata* (PDS) in each population separately. If the populations were in equilibrium these proportions would be expected to fluctuate from generation to generation and the distribution of the magnitude of these fluctuations should conform well to a binomial distribution with an N equal to the average population size. It does not. As estimated from the two figures, 160 of the 308 total weekly fluctuations (twenty-eight weekly periods times eleven populations) were of such magnitude that their probability of occurrence was less than 0.05. The testing was extremely conservative because it was based on an N of 500 which is lower than the average population size reported by Ayala, and the weekly intervals chosen are only 0.33 of the generation time he reports. This shows that these populations were not in equilibrium but that something was at work shifting the PDS first one way then the other. Furthermore, the period of fluctuation of the manipulative factor was a fraction of the generation time of the flies.

The manipulative factor exerted its effect on all replicates simultaneously, so most of the time the fluctuations of the populations were in the same direction. For example, during the 28 weeks under consideration 164 of the 308 total fluctuations (164/308=0.533) in the PDS were "ups". For each week the number of ups in all populations was totalled and the distributions of the totals (from 0–11) was examined. This distribution:

Class	0	1	2	3	4	5	6	7	8	9	10	11
Frequency	0	1	2	3	1	4	5	5	4	2	0	1

has a variance of 5.57, which is considerably larger than 2.74, the variance of a binomial distribution with $P=0.533$, $n=11$. A check on the confidence limits of the observed variance showed the probability that the true variance was as low as 2.74 to be less than 0.005. The manipulative factor must therefore be external to the populations. Limiting the analysis to the 160 "significant" fluctuations and comparing the two series (AR and CH) with respect to agreement or disagreement in majority trend (up or down) for each week, I found that there were sixteen agreements and two disagreements (for 10 of the weeks there was no majority trend in both of the series). On a binomial expectation of $P=0.5$ (to be expected if the trends of the two series were unrelated) the probability of this result is only 0.0007. This degree of synchronism is ample proof of the existence of an external factor.

The factor was probably ambient temperature. Ayala reported that the temperature of his incubators was controlled

Principle of Competitive Exclusion and *Drosophila*

THE Volterra–Gause equations¹—the mathematical formulation of the principle of competitive exclusion²—are

$$\frac{dN_1}{dt} = r_1 N_1 \left(\frac{K_1 - N_1 - \alpha N_2}{K_1} \right), \frac{dN_2}{dt} = r_2 N_2 \left(\frac{K_2 - N_2 - \beta N_1}{K_2} \right)$$

where r_1 is the intrinsic rate of increase of species 1, N_1 is its population size, and K_1 is the expected value of N_1 in single

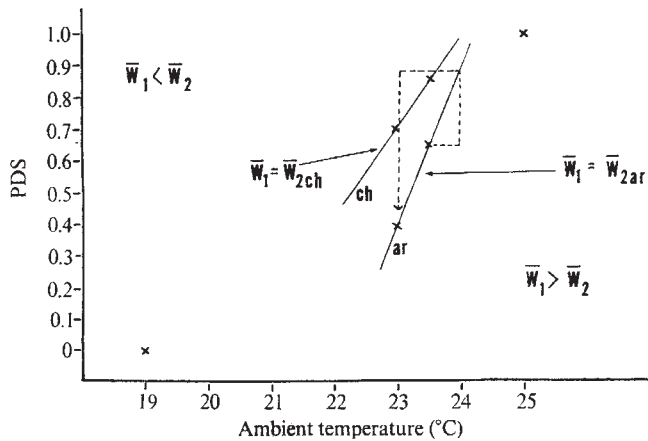


Fig. 1 Fitness relationships with respect to temperature and mixture of competing species. The ordinate is PDS or proportion of *D. serrata* in the cultures. The points are medians for data reported by Ayala³. $S_1 = D. serrata$, $S_2 = D. pseudoobscura$. The solid lines are drawn through the two points for each series and are the loci of all points where the two species have equal fitnesses. There are two lines because the species relationships differ with differences in genotype of S_2 (that is, CH or AR). The extrapolated portions of the lines are qualitatively correct. Below the lines $\bar{W}_1 > \bar{W}_2$ while above them $\bar{W}_1 < \bar{W}_2$. Any mixed population on its line is in equilibrium but other populations are subject to change in PDS because of selection. The dashed line traces the history of the hypothetical population of the text; in this representation, vertical movements are a selectional response to the horizontal movements.

to within 0.5 C min of the mean. His Figs. 1 and 2 show, however, that in this type of experiment this degree of control is inadequate. For example, a change of 0.5 C min in average temperature on week 23 caused a change in the PDS for the AR series from 0.65 to 0.39 (approximately) while in the CH series the shift was from 0.86 to about 0.70. In other words, the fluctuations in temperature permitted in the incubators (as much as a full centigrade degree) were great enough to have exerted large selective pressures.

A simple, testable model that incorporates the presence of fluctuating temperature accounts for the missing flies. When two competing species (S_1 and S_2) coexist in nearly constant proportions as in the case of Ayala's flies, the mean fitnesses (relative number of offspring left) of both species are equal, on the average through time. If this were not the case the proportion of S_1 would constantly trend in one direction and ultimately one species would be left. Indefinite coexistence implies that a balance point exists and, furthermore, that when the proportion of S_1 drops below that point the mean fitness of S_1 (that is, $\bar{W}_1$) exceeds the mean fitness of S_2 and when the proportion of S_1 rises above that point then $\bar{W}_2$ exceeds $\bar{W}_1$. If this were not the case the proportion of S_1 would drift and ultimately one species would be eliminated.

Ayala says that he has indirect evidence that the relative fitnesses of his fly species are frequency dependent in the manner just outlined. Further, the position of the balance point depends on temperature; and genotype affects both the position of the balance point and its sensitivity to temperature changes (Fig. 1).

Suppose a population of *D. pseudoobscura* AR/*D. serrata* in equilibrium at 23.5° C with a PDS of 0.65 is subjected to a temperature rise of 0.5° C. The population is then in disequilibrium and selection starts to alter the PDS so that it approaches 0.90. In the process, *D. pseudoobscura* flies are lost. After some time the temperature drops to 23.0° C and selection for a new balance point (PDS=0.39) commences. As a result, *D. serrata* flies are lost. Subsequent temperature fluctuations maintain a state of disequilibrium. The result of this process is to cause a lowering of mean population fitness and an excess loss of flies; this is the population size depression demonstrated by Ayala. The degree of the effect depends on the frequency

of the fluctuations. If they are relatively infrequent with respect to the generation time of the flies, a high ratio of recruitment to selective deaths will mask the effect. On the other hand, if they are relatively frequent the effect may be important. In these experiments the fluctuation period was of the order of one-third of the generation time.

What further evidence exists in Ayala's data for this interpretation? If temperature fluctuations are the prime cause of the missing flies, then that series in which the position of the balance point is most sensitive to temperature changes (*D. pseudoobscura* AR/*D. serrata*) should show the greatest depression in total population size. This is so. Units of one species may be coded in terms of the other by introducing a constant, C , so that $K_1 = CK_2$. Now, if there is no depression of numbers in the mixed population than K_1 should equal $N_1 + CN_2$. If K_1 exceeds $N_1 + CN_2$ then a depression exists and it can be measured by the size of B where $K_1 = N_1 + CN_2 + B$. I calculated the value of B for all populations. For the *D. pseudoobscura* AR/*D. serrata* populations the values (expressed as a proportion of K_1) were: 0.349, 0.378, 0.408, 0.419, 0.424, and 0.425. For the five *D. pseudoobscura* CH/*D. serrata* populations they were: 0.285, 0.330, 0.345, 0.350, and 0.412. The degree of depression was indeed greater in the AR series and the difference between the two sets of data has a one-sided chance probability of 0.05 (Wilcoxon's t test).

Moreover, if the depression of numbers was due to the factors I have mentioned, those populations that show the most evidence of having undergone selection should show the greatest depression of numbers. For an independent measure of sustained selection, I chose the total vertical distance travelled by each population in Ayala's Figs. 1 and 2. That is, I identified all the maxima and minima of PDS for a given population and summed the successive absolute differences to give me an index that combined both selectional and random factors. Assuming that random factors were about equal for all populations, differences in indices should reflect differences in sustained selection. If this assumption is unjustified, it serves merely to weaken the power of the test and make the conclusions more conservative. The populations in each series were then ranked according to their B values and selection indices. In both series the ranks were positively correlated, that is, the higher the selection index, the higher the B value. The Spearman's rank correlation coefficient (rd) for the AR series was 0.37, $N=6$, $P=0.25$, while for the CH series, $rd=0.60$, $N=5$, $P=0.18$; the two results evaluated with the Kolmogorov-Smirnov statistic have a joint probability of 0.12. When the data from the two series were combined and evaluated directly, rd was found to equal 0.486, $N=11$, $P=0.067$. This is as good a confirmation of my prediction as can be expected with so few data.

In summary, Ayala's experiments were an inadequate test of the Volterra-Gause equations because the necessary condition of stable equilibrium never obtained. I conclude that Ayala's rejection of the principle of competitive exclusion was unwarranted by his evidence. An alternative explanation for the discrepancies noted by Ayala was offered, tested, and found to be consistent with all the data.

This was written while I was at the Zoology Laboratory, University of Leiden.

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BOOK REVIEWS

Science and Humanity

Innovations: Scientific, Technological and Social. By Dennis Gabor. (Science and Engineering Policy Series.) Pp. vi+113. (Oxford University: London, November 1970.) £0.75.

Engineers and Engineering. By Lord Hinton of Bankside. (Science and Engineering Policy Series.) Pp. 75. (Oxford University: London, November 1970.)

"SCIENCE and technology have done more in the last ten decades to improve social conditions than the humanities have done in the last ten centuries," claims Lord Hinton as he concludes the first volume of this important new series. What the publisher hopes to achieve is to provoke those influential in shaping society into serious reflexion of the wide impact and consequences of the "science century". Future development may then, hopefully, be directed in the true sense, rather than lumbering onward, guided only by its own irresponsible momentum.

Lord Hinton launches the series on a somewhat disappointingly low key with a lamentation of the poor esteem in which the engineering profession is held by the intellectual community. This is no less than a tragedy, he asserts, because ultimately it is the engineer who actually fashions the fabric of society. Not only must engineering be an instinctively creative exercise ("engineering is not a science; it is an art"), but its practitioners must also be alive to the non-material needs of the society whose environment is being built. In a situation where the profession ranks as a mere second cousin to pure science, society has only itself to blame for self-inflicted injuries.

For the science and engineering community to be really effective, a partnership of respect needs to be created. Such a partnership exists in too few areas of technological endeavour, and Lord Hinton is clearly concerned by this. Much of the blame, he suggests, rests with the scientist, who too often keeps his eyes glued to the minutiae of academic problems rather than occasionally looking around to see just what is the context of his scientific labours.

In his much more stirring book, Dennis Gabor directs his venom at that

blindly materialistic system whose standard reads, "innovate or die". He contrasts the industrial society in its youth, when technological development without an explicit science base achieved important changes in man's material surroundings, with today when innovation has acquired a momentum in its own right, apparently unrelated to the real needs of society. Gabor lines himself up with the brave few who shout, "Stop, we are going the wrong way!" But he is no Luddite. "Growth addition," he says, "has harnessed inventive energies to blind material ends so that the scientist has become far removed from, and unaware of, the society which he is altering in dramatic ways." As the great innovative machine becomes increasingly efficient and remote, so man's ability to adapt to the fruits of his own efforts becomes dangerously stretched. The time lapse between discovery and commercial application seems steadily to be diminishing. One consequence is that significant changes in modes of living now occur well within man's natural time unit, a generation. Such a situation can only place increasing demands on man's finite ability to adapt.

It is time, Gabor argues, that we try to decide what is best for the quality of life rather than pursuing mindlessly what is best for the economics of growth. This is all good rousing stuff, closely argued with a controlled passion. Much of the book lists in cool, dispassionate detail, material, biological and social innovations possible in the next half century. These examples, fascinating in themselves, add force to an already persuasive argument.

ROGER LEWIN

Outlook of Relativity

Talking About Relativity. By J. L. Synge. Pp. 193. (North-Holland: Amsterdam and London; Elsevier: New York, March 1971.) \$5.75.

PROFESSOR SYNGE records that he chose the title before he wrote a word of the book. Clearly he chose it with deliberation, for he sees himself as meeting a challenge in communication. As nearly as is possible by means of the printed page, he does this by carry-

ing on a dialogue with his reader—hence "talking" in the title. Also, having a title at the start implies a well-defined aim, and Synge is here most certainly talking with a purpose. He is also talking with brilliance. He suggests, incidentally, that the inventor of zero should have got a Nobel prize in mathematics, had there been one; were there such a prize for expounding mathematics with a minimum use of mathematics it should undoubtedly go to Synge himself.

The book is addressed to anyone, layman or scientist, non-mathematician or mathematician, who wants to acquire the outlook of relativity in thinking about physics. While being diverted by Synge's sparkling presentation, he must be prepared to take trouble to grasp basic concepts. Synge offers him a succession of such concepts that are all essential to the purpose—the distinction between the R-world, the real world of experience, and M-worlds, the model or mathematical worlds of physical theory (Synge calls confusion between these the "Pygmalion syndrome"), concepts of algebra, geometry, function, operator, event, world-lines and the arrow of time, the concept of time itself, space-time and various associated concepts, including light-cones and curvature, the basic concepts of general relativity and its field equations (without writing them down except in symbolic form), the concept of gravitation, special relativity as a specialization of general relativity with some simple but fundamental applications.

Anyone who reads the book with attention—and anyone who reads it at all is certain to have his attention captured by it—no matter how little or how much he already knows, is bound to have his interest quickened and his ideas clarified and deepened. However, fully to appreciate the superb artistry of Synge's presentation, the reader should presumably know everything beforehand so that he can see what a wealth of thought has gone into the choice of every phrase (with apparently a curious solitary slip in describing a tensor transformation on page 143). Thus the book ought to prove equally invaluable to all who have to learn or to teach relativity.

W. H. MCCREA

Animal Systematics

Principles of Systematic Zoology. By Ernst Mayr. Pp. xi+428. (McGraw-Hill: New York and Maidenhead, 1969.) £6.00.

For many years, Ernst Mayr has been a leading American publicist in the struggle to maintain, in defiance of the zeitgeist, the central importance of systematics in the science of zoology. With the issue of the fight to which Mayr has devoted so much of his time and formidable energy still undecided either in the USA or in the world at large, many of us regret the lack of a champion of his stature to wage a similar battle in Britain. The present book, designed primarily as a practical handbook for those already committed to the pursuit of systematics, is fitted also to recruit to the side of Mayr's angels any uncommitted zoologist with an interest in the diversity of animals.

The book is in essence a rewritten and updated version of the well-known *Methods and Principles of Systematic Zoology* of Mayr, Linsley and Usinger (1953). That work has been out of print for some time and, respected as it was among professional systematists, it suffered from a rather severely pedantic style of presentation. The new book is far easier to read and, while sacrificing none of the valuable scientific content of its predecessor, incorporates much new material on nomenclature, numerical taxonomy, biochemical systematics and so forth.

In relation to recent disputes about the proper basis of classification, Mayr adopts a more or less pragmatic approach. While admitting the usefulness in some circumstances of the techniques of the numerical taxonomists, he rejects their fundamentalist claims (treating them as a variant of philosophical "nominalism"); similarly, he regards an understanding of evolutionary relationships as essential to the construction of a really natural classification, but holds up to ridicule the effects of a general application of Hennig's phylogenetic principles (known as "cladism" in America).

As a practising systematist, Mayr works with birds, and the characteristic bias of 20th century avian systematics—an overwhelming preoccupation with species and subspecies as against higher classification—is perceptible in his book. The two chapters on species and infra-specific classification are particularly good, whereas classification above the species level is rather summarily disposed of in only six pages. He states indeed that "the time has not yet come to present a well-balanced methodology of macrotaxonomy".

On the actual procedures of a modern museum systematist Mayr writes well

and with considerable authority. He is rather cautiously optimistic about the future possibilities of protein and DNA studies and of serotaxonomy, which are somewhat briefly considered in the book, but is fully alive to the importance of behaviour as a factor in animal evolution and hence in classification. Fossils, and the special problems involved in classifying them, are not specifically considered, no doubt in deference to the superior authority in this field of G. G. Simpson and A. S. Romer. The palaeontologist will probably continue to refer to Simpson's *Principles of Animal Taxonomy* (1961).

Mayr is an eminent member of the International Commission on Zoological Nomenclature, so it is not surprising to find in his book the complete English language text of the latest (1961) international code of nomenclature, together with an unusually extensive and authoritative exposition of its implications. Prospective purchasers of the official text of the code might get better value for their money by buying Mayr's book instead. It is understandable that he, unlike some other recent writers on the subject, refrains from radical criticisms of the present code.

In sum, this work offers perhaps the most representative and authoritative of all the accounts yet published of the principles and practices of present-day systematic zoologists, and for this reason may be valued by historians of science in future centuries. Mayr is not concerned to advocate views which are peculiarly his own, but writes responsibly as the spokesman of a rather solid consensus. Those who look for radically new ideas or approaches will not find them in this book, but should be warned that books in which such ideas are put forward generally fall a long way short of this one in other respects.

R. A. CROWSON

Light, Time and Animals

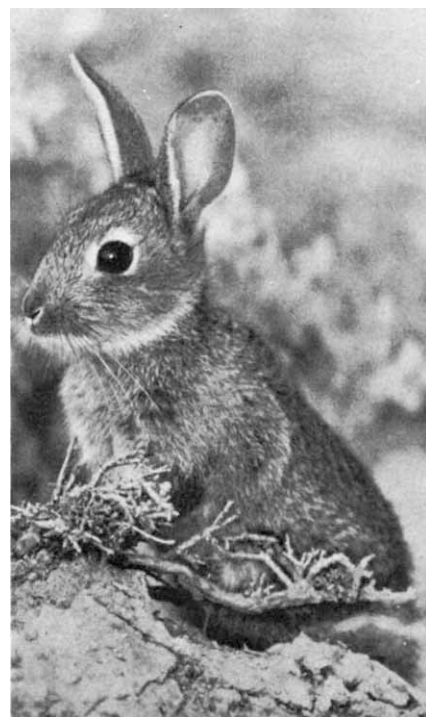
Animal Photoperiodism. By Brian Lofts. (The Institute of Biology's Studies in Biology, No. 25.) Pp. 62. (Arnold: London, November 1970.) £1.00 boards; £0.60 paper.

THE preface to this booklet states that it is an introduction to the extensive literature on photoperiodism aimed at students at school or in their first year at university. It certainly achieves this aim, and is an admirable attempt to bring together information on seasonal and daily cycles in all the animal groups which have been used as experimental material, although it naturally stresses the author's own interests in avian photoperiodism. This accent on birds makes the chapter on invertebrate

(meaning arthropod) photoperiodism seem a bit thin, especially as the preface also states that suggestions are made for appropriate practical work, and insects present very favourable experimental material in terms of speed of reaction, size and expendability. In the chapter on daylength measurement more could have been said about the number of light:dark cycles required to produce the photoperiodic response, and perhaps something on the location of the clock, its spectral sensitivity, and the endocrine mechanisms linked to it. Results from "night interruption" experiments with *Pieris brassicae* showing one peak of diapause inhibition are the result of incomplete "scanning" of the night by light pulses (Fig. 6—4), and other, two oscillator, hypotheses have been proposed to account for time measurement in the insects.

D. S. SAUNDERS

From the Camargue



This young rabbit (*Oryctolagus cuniculus*) is an inhabitant of the Camargue, that triangle of land bounded by the two arms of the Rhône and by the Mediterranean coast which forms the area of the Rhône estuary. Myxomatosis decimated the rabbit population of the Camargue in 1953, but more recently their numbers have recovered. This photograph is one of a splendid series both in colour and in black-and-white which illustrate *Camargue* by Karl Weber and Lukas Hoffman, the result of a four year ecological study of the region (Harrap: London, March 1971, £6.30).

Inside Plant Cells

Plant Cell Physiology: a Physicochemical Approach. By Park S. Nobel. (A Series of Books in Biology.) Pp. viii+267. (Freeman: San Francisco and Reading, January 1971.) £3.60.

THIS book has been written primarily for advanced undergraduate and graduate students of plant physiology and biophysics. Although the author has presented a fairly rigorous physicochemical approach in discussing plant cell processes, the reader does require some previous knowledge of elementary physical chemistry and calculus. The title is a little misleading, for the contents are essentially restricted to Nobel's own specialities, namely ion and water movement in plant cells and primary energy conversion processes of photosynthesis. It does not, however, include extensive experimental observations, but stresses the theoretical concepts which govern these processes.

There are six chapters, the first of which consists of a mixture of topics ranging from the basic morphological features of plant cells to Fick's laws of diffusion. Fortunately, the contents of the successive chapters have been more logically thought out. In fact, the second chapter gives an excellent description of the properties of water and its movement in plant cells and their organelles, as well as considering briefly specialized plant tissue such as the xylem and roots. This topic has been approached in terms of classical thermodynamics and the reader is introduced to quantities such as chemical and water potential. This thermodynamic approach is continued in the following chapter, which is partly concerned with the principles governing the passive distribution in, and flux of charged solutes between, various compartments of plant cells. For example, the concepts which give rise to the derivation of such expressions as the Nernst equation, the Goldman equation and the Ussing-Teorell equation are explained. This chapter on solutes also includes a short section on active transport and an introduction to the basic concepts of irreversible thermodynamics emphasizing particularly how reflexion coefficients can be used to modify classical equations such as the Boyle-Van't Hoff relation.

The remaining chapters are essentially concerned with energy conversion in plant cells, with particular reference to photosynthesis. A complete chapter is devoted to elementary photochemistry and, as such, gives a reasonable backing for the following chapter which deals with primary photosynthetic mechanisms. There is, for example, a detailed description of the optical and chemical properties of chlorophyll coupled to a section on

excitation transfer and trapping. The remaining part of the chapter outlines current ideas on electron transport, but does not deal with the biochemistry of carbon fixation. Finally, the last chapter considers briefly the thermodynamics of both chloroplast and mitochondrial energetics.

As far as I am aware there is no other textbook available which covers these topics of plant physiology and also deals with their physicochemical aspects. Consequently Nobel has made available an excellent and needed textbook for those who teach in this field. The earlier chapters on water and solutes are particularly valuable, although the section on photosynthesis does nothing more than present information which can be readily found in other recently published textbooks specifically written on this subject. The value of the book for teaching purposes is increased by the numerical problems at the end of each chapter, which have been designed to give the student an opportunity to apply the principles and equations presented in the text.

J. BARBER

Physiology of Emotion

Physiological Correlates of Emotion. Edited by Perry Black. Pp. xvi+309. (Academic: New York and London, October 1970.) £6.30.

THIS book consists of seven papers delivered at a conference held in Baltimore, supplemented by six contributions invited by the editor. The list of contributors is impressive, with leading American experts covering a wide range of physiological and psychological aspects of the problem. It is perhaps surprising to note that there is no clinical psychiatrist contributing. This may be because none could be found with the necessary depth of neurophysiological knowledge to survive in this company. Nevertheless, it perhaps indicates the gap which at present exists between those involved in basic neurobiological research and the practitioners who are treating emotional disorders, and throws some doubt on the statement about the book made on the dust cover, "... it also serves as a useful stepping-stone toward the management and control of disordered emotional states". Papers such as those by McLean on "The Limbic Brain in Relation to the Psychoses" and Lindsley on "The Role of Nonspecific Reticulo-Thalamo-Cortical Systems in Emotion" should certainly narrow this gap. Both authors present a clear authoritative review of their subject and are also not afraid of speculation regarding the changes to be found in emotional disorders, thus providing a stimulus to further discussion

and research. The paper by Harlow and Harlow on "Developmental Aspects of Emotional Behaviour" should also be singled out as a clear and stimulating review of original work in the field.

While other papers in the collection provide useful information and stimulating discussions, I felt that some of them, at times, had fallen into the trap of presenting too much in the way of detailed results which are to be found more appropriately in the papers of the appropriate scientific journal. In talking of emotion it seems that the contributors were most at home when considering arousal, fear and aggression. There is less material in the book dealing with depression or elation, although the contribution by Kety deals with some of the pharmacological aspects, and the paper by Dalgado on "Modulation of Emotions by Cerebral Radio Stimulation" also touches the subject.

In summary, the book provides a good introduction at a reasonably advanced level to the study of the physiology of emotion. The problems are not minimized and the reader should be stimulated to continue his own studies, realizing there is still much new country to be explored.

GEORGE W. ASHCROFT

Pictures Computed

Computer Techniques in Image Processing. By Harry C. Andrews. (Academic: New York and London, 1970.) \$10.50; £4.90.

THE problems of transmission, storage and classification of pictures are receiving more and more attention from scientists and engineers. The analysis of the redundancies in the pictures is often complicated by noise, spurious distortions or translations. Physicists have known for a long time that real space is not always the most appropriate one for the analysis of phenomena: the achievements of quantum mechanics and electronic engineering are, to a great extent, derived from the idea that the phenomena are better studied if projected on a proper set of coordinates—time or frequency for electronic signals, space or momentum for quanta of energy. The extension of this concept to pictures is traditionally known as Fourier optics. Recently, the decomposition of pictures onto orthogonal functions other than sine and cosine has opened new horizons for this kind of technique. Particular interest has been shown in functions which can be implemented simply on digital computers.

The author, H. C. Andrews, and his collaborators, W. K. Pratt and K. Caspari, have been known for many years as productive contributors to the

progress of computer techniques for orthogonal transformations, including the Fourier transform. Their expertise is a guarantee of first-hand accounts of the latest developments in these techniques.

The subject is treated with practical applications and implementation in mind. This makes the book valuable for both scientists and engineers dealing with transmission or compression of large quantities of data.

The presentation is concise (about 180 pages) but includes discussion and theoretical development when appropriate. Special tribute must be paid to the author's excellent choice of highly informative illustrations.

The topics covered are chiefly concerned with the processing of two-dimension pictures in the transformed domain, either through purely optical techniques or through various computed orthogonal transformations (discrete Fourier, Hadamard and so on). Possible applications to pattern recognition and economical coding of signals are suggested. Sampling techniques in the transformed domain and their effect on the reconstructed image are considered in some detail. The generous bibliography gives the reader a good introduction to further study of specialized applications.

The examples chosen reflect the experiments of the authors with photographs from space probes. Their conclusions, however, can easily be extended to any field where photographs or graphical patterns are processed (characters or pattern recognition, aerial photographs, biological and medical research, geological and sociological surveys and complex multivariate statistics among others) and wherever optical Fourier transform techniques can be enhanced or simulated on binary computers.

J. A. G. HALÉ

Nonlinear Problems

Iterative Solution of Nonlinear Equations in Several Variables. By J. M. Ortega and W. C. Rheinboldt. (Computer Science and Applied Mathematics: a Series of Monographs and Textbooks.) Pp. xx+572. (Academic: New York and London, August 1970.) £11.20.

THIS book is a result of five years' work by the authors and several PhD students at the University of Maryland with the intention of producing a text for postgraduate courses and a reference source for research workers.

The system under consideration is a set of n real nonlinear equations in n variables with isolated real solutions, which occurs in many fields of research such as nonlinear partial difference

equations and nonlinear multivariate regression analysis.

Approximately 90 pages are devoted to basic mathematical requirements in algebra and analysis, 90 pages to theorems on the existence of solutions, 100 pages to the description of major iterative methods, and 240 pages to the analysis of convergence. Considerable reference is made to the methods of Newton and successive over-relaxation, which have been treated in books by Ostrowski (1966) and Varga (1962).

Although this is indeed a comprehensive textbook, its scope is necessarily limited in several respects. There is no discussion of iterative methods which require the evaluation of second or higher derivatives; infinite dimensional spaces are not considered; no numerical examples are given; and there is no treatment of computational error. All these omissions are justified by the authors, who give references to other works which deal with these topics. Ill-conditioned equations, however, do not appear to merit comment. Partly as a result of these limitations, it is likely that this book will prove to be of greater use to postgraduate mathematics students, and their lecturers and supervisors, than to physicists and engineers engaged in the solution of specific problems.

The above remarks are not intended to detract from the value of this book. The presentation is admirably lucid, and the publishers are to be commended for an excellent standard of production. The book contains useful exercises, numerous informative notes and remarks, a very extensive bibliography, and author and subject indices.

A. FONDA

Guide to Examiners

The Principles of Objective Testing in Physics. By J. G. Houston. (The Principles of Objective Testing Series.) Pp. vii+102. (Heinemann: Educational: London and Edinburgh, August 1970.) £1.25.

THE author of this book has clearly had in mind that his readers will be more or less skilled already as examiners, and receptive in varying degrees to the suggestion that improvement in their present practice is possible. He accordingly assumes little constructive thought about the aims of examining, and so inevitably includes a great deal which many of those who use the book will regard as inapplicable to themselves. In fact, few of them will not gain by following through his clearly developed thesis, which is prepared to emphasize as strongly what objective testing, even at its best, is not fitted to do as what it can do well. It quickly becomes apparent that an effective introduction

to objective testing gains very much by being linked to the problems of a particular subject. But equally, even within that subject, although this book is directly aimed towards those teaching in the schools, it will undoubtedly provide helpful guidance to people also who have to examine and test at other levels. The book bears much evidence of care in its preparation, and this has certainly been rewarding.

J. G. WILSON

Crystal Structures

Crystals and X-rays. By H. S. Lipson. (The Wykeham Science Series, 13.) Pp. xiv+198. (Wykeham: London and Winchester, October 1970.) £1.75.

THE avowed aim of the Wykeham Science Series is "To introduce the present state of science as a university subject to students approaching or starting their university careers . . .". The problem is how, on the one hand, to avoid an erudite exposition while, on the other hand, not producing too sketchy an outline. Crystal structure, and the mechanics of its determination, is not an easy subject.

The book starts from the optical microscope and leads on to the wave theory, Abbé theory and the limit of resolution. From this, consideration is given to X-rays, their discovery, production, properties and uses in the determination of crystal structure. The treatment of this is in historical order, includes examples of the determination of actual structures and covers all the principal techniques in use today. Of necessity, in a volume of this size, the development of the subject proceeds very fast. I wonder if the uninitiated can be expected to cope with the rate at which ideas and terminology are introduced without recourse to a lot of outside references or to the help of a sixth form master well versed in the subject of crystallography (and how many are?). The subject matter is treated more than superficially, Miller indices being introduced perforce and reciprocal lattices and Fourier methods being just two of the topics that students could find difficult.

The later chapters deal in an interesting manner with discoveries made by X-ray diffraction methods and the technological uses of X-rays. It seems a pity that many sixth form readers will not read these chapters, through having become bogged down in some of the rather heavy work earlier on.

The book is lavishly illustrated with X-ray photographs, diagrams and the like, is well cross referenced and has a good index. It deserves a place on the shelves of any sixth form library but the student may need some encouragement with the middle chapters.

D. A. TURNER

Obituary

Professor D. G. Millar

DAVID GAVIN MILLAR, Foundation Professor of Human Reproduction and Obstetrics at the University of Southampton, died on March 4 at the age of forty-one. The son of a general practitioner, he was born in Durham and educated at Durham School. He studied medicine at the University of Durham (at Newcastle upon Tyne), graduating with honours in 1952. He was elected FRCS in 1959 and MRCOG in 1962.

As an undergraduate he won the Gibson Prize in Obstetrics and the British Medical Association essay competition for medical students in 1952—the subject of his essay was “The Training of the Student in Personal Relationships between Doctor and Patient”. After a distinguished undergraduate career and service in the RAMC he returned to Newcastle upon Tyne where he trained first as a general surgeon and then as a gynaecologist. He soon secured the reputation of a highly skilled and sympathetic clinician and on this sound foundation he built his research interests. As Luccock Research Fellow from December 1959 to March 1961 he mastered the use of the digital computer and produced programs for the handling

of data from the community study of obstetrics in the City of Newcastle upon Tyne. This personal experience of the application of computer techniques to research in obstetrics led to his taking a serious and lasting interest in the application of the computer to a wide variety of problems in medicine and to his appointment to a number of important medical committees. One of the highlights of the 17th British Congress of Obstetrics and Gynaecology in 1965 was his paper on the contribution of the computer to research in obstetrics. David Millar was one of the few medical men who really understood and could talk authoritatively on the subject. He also contributed notably to the success of the new undergraduate curriculum in Newcastle upon Tyne. He understood the need to break away from the narrow, “departmental” approach to teaching with its tendency to present medicine as a series of disjointed and unrelated specialties. He was, in fact, a strong supporter of the integrated pattern of teaching and argued convincingly that human reproduction, touching on so many disciplines, lent itself to this approach. In Newcastle he helped build up a library of objective multiple choice questions for use in the final examinations and his complete grasp of the computer

analysis of such questions led in time to his appointment as chairman of the committee appointed by the Royal College of Obstetricians and Gynaecologists to supervise the setting and marking of the new MRCOG (Part 1) multiple choice question paper.

A major research interest, which extended over many years, was the effect of certain obstetric events on the physical and intellectual development of children. Here he collaborated principally with Dr Gerald Neligan in the Department of Child Health and several important papers were delivered to societies in the United Kingdom and America. He showed, for example, that such obstetric complications as vaginal breech delivery and placenta praevia had a significantly adverse effect on the IQ of surviving children at the age of 5 years.

His recent appointment to the chair in Southampton seemed to set the seal on what was to be a brilliant career and his tragic death at such an early age is a grievous loss to the subject and Southampton. Already David Millar had established a national reputation and had he lived he would certainly have reached the front rank of his chosen specialty. He is survived by his wife (also a doctor), two sons and twin daughters.

Announcements

University News

Professor Gordon Atherley has been appointed to the newly established chair of safety and hygiene in the **University of Aston in Birmingham**.

Dr E. N. Corlett has been appointed to the newly established chair of industrial ergonomics in the **University of Birmingham** and **Dr D. G. Walker** has been appointed to a chair of biochemistry.

Dr C. Andrew has been appointed to the chair of mechanical engineering, **Dr J. F. Mitchell** to the chair of pharmacology and **Dr R. D. Milne** to the chair of theoretical mechanics, all in the **University of Bristol**.

Dr D. J. Osborne, deputy director of the ARC Unit of Developmental Botany, has been elected to a fellowship at Churchill College, **University of Cambridge**.

Sir Frederick Warner has been appointed chairman of the council of the **School of Pharmacy, University of London**, in succession to **Sir Harry Jephcott**.

Mr L. C. B. Gower will succeed **Professor Kenneth Mather** as vice-chancellor of the **University of Southampton**.

Professor T. A. Bennet-Clark, University of East Anglia, has been appointed Royal Society visiting professor in the Department of Botany, **University of Zambia**.

Miscellaneous

Dr George R. Hill, dean of the College of Mines and Mineral Industries at the University of Utah, will be the 1971 recipient of the H. H. Storch award for research on coal chemistry, presented by the Fuel Division of the **American Chemical Society**.

International Meetings

May 24, **Environmental Influences on Reproduction**, London (Dr R. E. Lister, Inveresk Research International, Inveresk Gate, Musselburgh, Midlothian).

May 31–June 2, **Canadian Chemical Conference**, Halifax, NS (The Chemical Institute of Canada, 151 Slater Street, Room 906, Ottawa 4, Ontario, Canada).

June 6–11, **European Rheumatology Congress**, Brighton (Congress Secretariat, c/o Arthritis and Rheumatism Council, 8–10 Charing Cross Road, London WC2).

June 10–11, **Metabolism and Disease**, Ottawa (Dr R. S. Meldrum, Food and Drug Directorate, Tunney's Pasture, Ottawa 3, Ontario, Canada).

July 5–9, **Introductory Course in Tropical Hygiene**, London (Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel Street (Gower Street), London WC1E 7HT).

July 8–9, **Behavioural Aspects of Parasite Transmission**, London (The Executive Secretary, Linnean Society of London, Burlington House, Piccadilly, London W1V 0LQ).

August 16–28, **Organizations and Computers**, Dubrovnik (L. Radanovic, Centre of Advanced Studies, PO Box 356, Belgrade, Yugoslavia).

September 9–12, **Taxonomy and Phytogeography of Higher Plants in Relation to Evolution**, Manchester (Dr C. A. Stace, Department of Botany, University, Manchester M13 9PL).

September 13–16, **Liquid Scintillation Counting**, Brighton (Mr M. A. Crook, Society for Analytical Chemistry, 9–10 Savile Row, London W1X 1AF).

September 14–16, **Quinone Chemistry**, Aberdeen (Dr L. Batt, Department of Chemistry, University of Aberdeen, Mes-ton Walk, Aberdeen AB9 2UE).

October 13–18, **The Protection of Nature and its Environment**, Rouen (Protechna-COMET, 48 Quai de Paris, 76 Rouen, France).

British Diary

Tuesday, April 13

Styling for Motor Vehicles (7.30 p.m.) Mr D. Bache, Society of Environmental Engineers, in the Mechanical Engineering Department, The University, Birmingham.

Tension Levelling (6 p.m. discussion) Institution of Mechanical Engineers, Applied Mechanics Group, jointly with the South Wales Branch, at Swansea.

Wednesday, April 14

Current Research in Crystallography (three-day conference) Institute of Physics and the Physical Society; and the Chemical Society, at the University of Manchester Institute of Science and Technology, Sackville Street, Manchester.

Electrical Conduction in Organic Solids (general discussion, three days) Faraday Society, in the Chemistry Department, University of Nottingham, University Park, Nottingham.

Externally Pressurized Bearings (two-day conference) Institution of Mechanical Engineers, Tribology Group, jointly with I.Prod.E., at Coventry.

Thursday, April 15

Development of Electrical and Electronic Systems during Flight Testing of New Aircraft (6 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Thermal Analysis with special reference to Microtechniques (two-day meeting) Society for Analytical Chemistry, at the Nobel Division, ICI Ltd, Ardeer, Ayrshire.

Friday, April 16

Engineering Science in Schools (2 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Effective Training of Professional Engineers as Managers (5.30 p.m.) Mr P. H. L. Thomas, Institution of Electrical Engineers, at Savoy Place, London WC2.

Monday, April 19

HV Overhead Transmission Lines (6.30 p.m.) Mr G. Orawski, Institution of Electrical Engineers, London Graduate and Students Section, at Savoy Place, London WC2.

On the Relativity of Time (5.30 p.m.) Mr A. Trieder, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

Semiconductor Circuit Design (vacation school, five days) Institution of Electrical Engineers, at the University College of Swansea, South Wales.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

Teachers and Youth Workers: a Study of Their Roles. (Schools Council Working Paper No. 32.) Pp. 128. (London: Evans/Methuen Educational, 1971.) 57p net. [153]
The Carnegie United Kingdom Trust. Fifty-seventh Annual Report, 1970. Pp. viii+76. Dunfermline, Fife: The Carnegie United Kingdom Trust, 1971. [153]
Fabian Tract No. 406: Divide and Rule: The Industrial Relations Bill. By Giles Radice and J. O. N. Vickers. Pp. 20. (London: Fabian Society, 1971.) 15p. [153]
Flora of Tropical East Africa. Edited by E. Milne-Redhead and R. M. Polhill. Hamamelidaceae. By B. Verdcourt. Pp. 5. 10p. Flagellariaceae. By D. M. Napper. Pp. 3. 10p. Juncaginaceae. By D. M. Napper. Pp. 3. 10p. Lecythidaceae. By G. R. W. Sangai. Pp. 6. 10p. Phytolaccaceae. By R. M. Polhill. Pp. 8. 15p. Oxalidaceae. By Christine H. S. Kabuye. Pp. 21. 25p. Geraniaceae. By J. O. Kokwaro. Pp. 23. 25p. Plantaginaceae. By B. Verdcourt. Pp. 7. 15p. Cabombaceae. By B. Verdcourt. Pp. 3. 10p. Typhaceae. By D. M. Napper. Pp. 5. 10p. Annonaceae. By B. Verdcourt. Pp. 132. 100p. (London: Crown Agents for Oversea Governments and Administrations, 1971.) £5.25; \$13. [163]
Curriculum Innovation in Science. By Professor K. W. Keohane. (Inaugural Lecture 4th November, 1969.) Pp. 11. (London: Chelsea College, University of London, 1971.) [163]

Heriot-Watt University, Edinburgh. Annual Report 1969/1970. Pp. 86. (Edinburgh: Heriot-Watt University, 1971.) [163]
University of Hull. Occasional Papers in Geography. No. 9: A Scheme for Hillslope Analysis. 1: Initial Considerations and Calculations. By A. F. Pitty. Pp. ix+76. 75p. No. 17: A Scheme for Hillslope Analysis. 2: Indices and Tests for Differences. By A. F. Pitty. Pp. viii+56. £1. (Hull: The University, 1969 and 1970.) [163]
Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates. By J. M. Elliott. (Scientific Publication No. 25.) Pp. 144. Ambleside: Freshwater Biological Association, 1971. 95p. [163]
Chatham House and PEP. European Series No. 16: Agricultural Policy and the Common Market. By John Marsh and Christopher Ritsen. Pp. 199. (London: Chatham House and PEP, 1971.) £1.25. [163]
University of Oxford. Lord Nuffield's Benefaction for Advance of Medicine—Report for 1968/69. Pp. 6. (Supplement No. 2 to the *University Gazette*, Vol. ci, Jan. 1971.) (Oxford: The University, 1971.) 25p. [163]
Tate and Lyle, Ltd. Group Research and Development Annual Report for 1970. Pp. 71. (Keston, Kent: Tate and Lyle, Ltd, 1971.) [163]
Energy Resources for Western Europe: Present Situation and Future Prospects. Pp. 37. (London: National Coal Board, 1971.) 75p. [173]
Abstraction in Science and Morals. By Professor Stephan Körner. (The Twenty-fourth Arthur Stanley Eddington Memorial Lecture delivered at Cambridge University, 2nd February, 1971.) Pp. 34. (London: Cambridge University Press, 1971.) 30p; \$0.95. [173]
Science Research Council. Report of the Working Party on High Temperature Processes. Pp. 36. (London: Science Research Council, 1971.) *Gratis*. [173]
Science Research Council. Report of the Working Party on Desalination. Pp. 40. (London: Science Research Council, 1971.) *Gratis*. [173]
Aslib. Occasional Publications. No. 6: The Distribution of Scientific and Technical Libraries and Users in Great Britain. By Alexandra Presanis. Pp. 32. £1.40. No. 7: Quantitative Data in Science and Technology. By Brenda Mountstephens, Averil Osborn and Margaret Slater. Pp. 24. £1.24. (London: Aslib, 1971.) [183]
Department of Trade and Industry; Ministry of Defence; and Joint Services Non-Metallic Research Board. A Review of the Science of Fibre Reinforced Plastics. By Dr N. G. McCrum. Pp. ix+140. (London: HMSO, 1971.) £1.90. [183]
National Museum of Wales. Sixty-third Annual Report 1969/1970. Pp. 135. (Cardiff: The Museum, 1970.) [183]
Race and Employment: Managing a Multi-racial Labour Force. By David Wainwright. Pp. 88. (London: Institute of Personnel Management, 1970.) £1. [183]
Six Great Goals. By President Nixon. (State of the Union 1971, delivered to the US Congress, January 22, 1971.) Pp. 15. (London: US Information Service, 1971.) [183]
The Geological Survey of Ireland. Bulletin No. 1, November 1970. Pp. 71. (Dublin: Department of Industry and Commerce, Geological Survey of Ireland, 1970.) [183]
Department of Education and Science. Statistics of Education 1969. Vol. 4: Teachers. Pp. xviii+83. (London: HMSO, 1971.) £1.70 net. [183]
Pollution Research and the Research Councils. Pp. 29. (London: Agricultural Research; Medical Research Council; Natural Environment Research Council; Science Research Council; Social Science Research Council, 1971.) [193]
Experimental Work 1969. Pp. viii+101. (Edinburgh: The Edinburgh School of Agriculture, 1970.) 37½p. [193]
The Scottish Field Studies Association, Ltd. Annual Report for 1970. Pp. 40+2 plates. (Blair Drummond, By Stirling: Scottish Field Studies Association, 1971.) 35p. [193]
In the Public Interest: a Six-Part Explanation of BBC Policy. Pp. 27. (London: BBC, 1971.) [193]
Institute of Linguists. Annual Report and Accounts for the year ended 31st July, 1970. Pp. 17. (London: The Institute of Linguists, 1971.) [193]

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